POTENTIAL BIOFOULING STRATEGIES AGAINST BLUE MUSSEL *(Mytilus Edulis)* INFESTATION IN A COOLING WATER SYSTEM

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By

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TABLE OF CONTENTS

Page

LIST OF TABLES iv

LIST OF FIGURES v

ABSTRACT vi

INTRODUCTION 1

THE BLUE MUSSEL (*MYTILUS EDULIS*) 2

BACKGROUND 2
LIFE CYCLE 4
FACTORS AFFECTING LARVAL DEVELOPMENT AND SETTLEMENT 6

CONTROLLING MUSSEL ACCUMULATION - ANTIFOULING 8

INTRODUCTION 8
CHEMICAL ANTIFOULANTS: OXIDIZING AGENTS 9
CHLORINE 9
CHLORAMINE 15
BROMINE 16
BROMAMINE 16
BROMOCIDE 17
BROMINE CHLORIDE 17
CHLORINE DIOXIDE 18
HYDROGEN PEROXIDE 19
HYDROGEN PEROXIDE + IRON 20
IODINE 21
OZONE 21
POTASSIUM FERRATE 22
POTASSIUM PERMANGANATE 24
CHEMICAL ANTIFOULANTS: NONOXIDIZING AGENTS 25
AMMONIA & AMINES 25
CARBAMATE COMPOUNDS 28
CYANO COMPOUNDS 29
METAL SALTS 31
ARSENIC 31
CADMIUM 31
CHROMIUM 33

TABLE OF CONTENTS

Page

COPPER 33
LEAD 36
MANGANESE 37
MERCURY 37
NICKEL 38
POTASSIUM 39
SELENIUM 39
SILVER 39
ZINC 40
MOLECULAR REGULATORS 43
NATURAL PRODUCTS & ANALOGS 44
PAINT ADDITIVES: ORGANOMETALLICS 47
PAINT ADDITIVES: OTHERS 47
NONCHEMICAL ANTIFOULANTS 50
THERMAL 51
NONTHERMAL ENERGY 53
ACOUSTIC ENERGY 53
ELECTRICAL 53
ELECTROCHEMICAL 55
GAMMA IRRADIATION 57
ULTRAVIOLET RADIATION 57
ULTRASONIC VIBRATION 57
HYDRAULIC 58
WATER JET CLEANING 58
WATER VELOCITY 58
MECHANICAL 59
AMERTAP 59
AMERICAN M.A.N. 59
PIPE ROBOT 60
MISCELLANEOUS 60
ANOXIC WATER 60
LOW ENERGY COATINGS 61
NEW PAINT FORMULATIONS 63
OSMOTIC SHOCK 64
COMBINATION OF TREATMENTS 65
INTERNET PRODUCTS 67
BIOFILM 67
SUMMARY 69

REFERENCES 73
INTERNET SITES 89

LIST OF TABLES

Table Page

1. Possible methods of controlling mussel infestation....10

2. Oxidizing agents - chlorine (Cl_2, HOCl, etc.)....13

3. Oxidizing agents - excluding chlorine....23

4a. Nonoxidizing agents - organics....30

4b. Nonoxidizing agents - metal salts....41

4c. Nonoxidizing agents - natural products and analogs....46

4d. Nonoxidizing agents - paint additives: organometallics....48

4e. Nonoxidizing agents - paint additives: others....49

5. Thermal methods....52

6. Miscellaneous - low energy coatings....62

7. Summary....71

LIST OF FIGURES

Figure Page

1. Diagram of the mussel showing the location of the principal organs. 3

2. Morphology of the byssus. 4

3. Generalized life cycle of blue mussel, *Mytilus edulis*, in eastern Canada. 5

ABSTRACT

This report reviews the past and recent literature for controlling mussel biofouling in cooling systems. Treatment selection and factors (biological, chemical, and physical considerations) affecting mussel infestation are discussed comprehensively. In the use of chemical or nonchemical methods, related problems such as toxicity to non-target organisms and the environment, and/or financial costs are extensively incorporated into the report. Other antifoulant methods such as thermal, electrochemical, irradiation, ultraviolet, hydraulic, biofilm, and robot mechanic cleaning are also included in the discussion. The objective of this report is to aid in the understanding and the choice of effective, useful, and inexpensive solutions for biofouling problems.

RÉSUMÉ

Ce rapport fait le point sur les publications passées et présentes concernant la lutte contre la colonisation par les moules des systèmes de refroidissement. Il examine de façon approfondie le choix des traitements et les facteurs (considérations biologiques, chimiques et physiques) qui agissent sur l'infestation par les moules. Dans le cas des méthodes chimiques et non chimiques, les problèmes connexes comme la toxicité pour les organismes non ciblés et l'environnement, et/ou les coûts financiers, sont exposés en détail. D'autres méthodes antisalissures comme le traitement thermique ou électrochimique, l'irradiation, les ultraviolets, les techniques hydrauliques et le nettoyage par robot mécanique sont également abordées. L'objectif du rapport est d'aider à comprendre et à choisir des solutions efficaces, utiles et peu coûteuses aux problèmes de salissures.

INTRODUCTION

The natural process of marine biofouling has proven to be a stubborn problem for ocean traveling vessels. Historical records indicate it's occurrence over many centuries (Field, 1981). Problems affiliated with biofouling still plague the shipping industry, and many other industries that make use of either fresh or saltwater.

The problems associated with biofouling are numerous. The most obvious and best researched example is the biofouling of the hulls of ships. Biofouling also causes great difficulties for industries that use raw water for cooling purposes. Biofouling not only creates heat exchange problems, but also causes restriction of flow through the cooling water system. This decreases the efficiency of the system and may ultimately result in system failure.

The extremely complicated process of biofouling is still not well understood. However, in recent years, a greater effort has sought to understand the processes involved, and current knowledge indicates that biofouling progresses in stages: (1) absorption of dissolved chemical compounds to the object surface, which occurs within seconds of immersion; (2) bacterial colonization (slime film or microfouling) which begins several hours after initial immersion; (3) the colonization of yeasts, protozoa and diatoms; (4) the settlement of planktonic larvae and algal spores (macrofouling) which is the last and longest phase (Wahl, 1989).

Many variables (i.e. temperature, chemical content, native organisms, substrate, nutrient supply, etc.) are involved in every different biofouling situation. Therefore, what works in one situation, may not be sufficient elsewhere. Each problem must be examined individually, and the significant variables associated with each location must be considered. The knowledge and understanding of these variables will increase the chances of determining the proper solution.

The aim of this report is to review the literature for all and any potential solutions for biofouling problems, with emphasis on those designed to prevent the settlement and growth of the blue mussel (*Mytilus edulis*) in a cooling water system. The life cycle of the blue mussel and its related biological processes will also be described. Existing and developing methods for biofouling control will be discussed in relation to the problem organism; i.e., regarding the relative effectiveness, suitability, cost and concerns involved with each.

The global costs associated with the control of biofouling are enormous. To control these costs, many different techniques have been employed over the years with varying results. Many have proven to be ineffective, while others were too costly. Some of the successful, currently used methods, are coming under greater scrutiny because of

their environmental effects. The largest concern is the consequence of biofouling control on non-target organisms. It is apparent that no single solution will solve all the existing biofouling problems.

THE BLUE MUSSEL (*MYTILUS EDULIS*)

BACKGROUND

Mussels are bivalve molluscs belonging to the family Mytilidae. The common or blue mussel, *Mytilus edulis*, is among the most abundant and widely distributed invertebrate species inhabiting intertidal and shallow subtidal waters in the North Atlantic (Stewart, 1994). It is also found in Arctic waters, as far north as Greenland, and its southern distribution extends to South Carolina on the Atlantic coast. *M. edulis* also inhabits the Pacific coast as far south as California and west to Japan, as well as in European waters as far south as the Mediterranean and North Africa. Range limitations seem to be governed mainly by temperature. Although mussels are able to survive freezing for extended periods, elevated temperatures of about 27°C (surface) limit their southern distribution (Seed, 1976).

The widespread distribution of *M. edulis* has lead to extensive research on this marine organism. For example, *M. edulis* is one of the most commonly used (if not the most common) species for studies of contaminant toxicology and physiological effects. Also, it has been used thoroughly in environmental impact and assessment studies (Calabrese et al., 1984), and is the main organism used in *Mussel Watch* studies world wide, for evaluating chemical contamination, and identifying trends in environmental contamination. Mussels can tolerate many chemical contaminants (at higher concentrations than many organisms) and with their fairly wide range tolerance to temperatures and salinities, makes this species the ideal candidate for such studies.

Adult mussels are sessile animals that attach to hard substrates by way of byssal threads, produced by the byssal gland. This gland is located just behind the foot, (see Figure 1) which aids the byssal gland in byssal attachment. Many (up to several hundred) of these byssal threads are secured in a radial pattern to the substrate to resist current and wave action. These threads are composed of protein fibers connected to a disk-shaped adhesive plaque that adheres to the substrate (see Figure 2). Byssal threads are amazingly resilient, combining mechanical properties of considerable strength and shock resistance. A detailed description of byssal thread function and composition is given by Smeathers & Vincent (1979) and Rzepecki & Waite (1995).

After the mussel (pediveliger) has found an adequate site for settlement and attaches to the substrate via byssal threads, it begins to feed and grow. It feeds by filtering suspended phytoplankton and detrital organic matter from the surrounding water. Water is drawn into the mussel through the inhalant siphon and passes through the gills. Food particles are trapped by the gills and passed onto the mouth. From the mouth, the food particles pass through the oesophagus and enter the stomach. After some further sorting, the food travels to the digestive gland (Bayne et al., 1976). Provided that conditions are conducive to growth (temperature, salinity, nutrient supply, competition, etc.), the mussel will reach it's adult size (6-10cm) in 1 to 3 years.

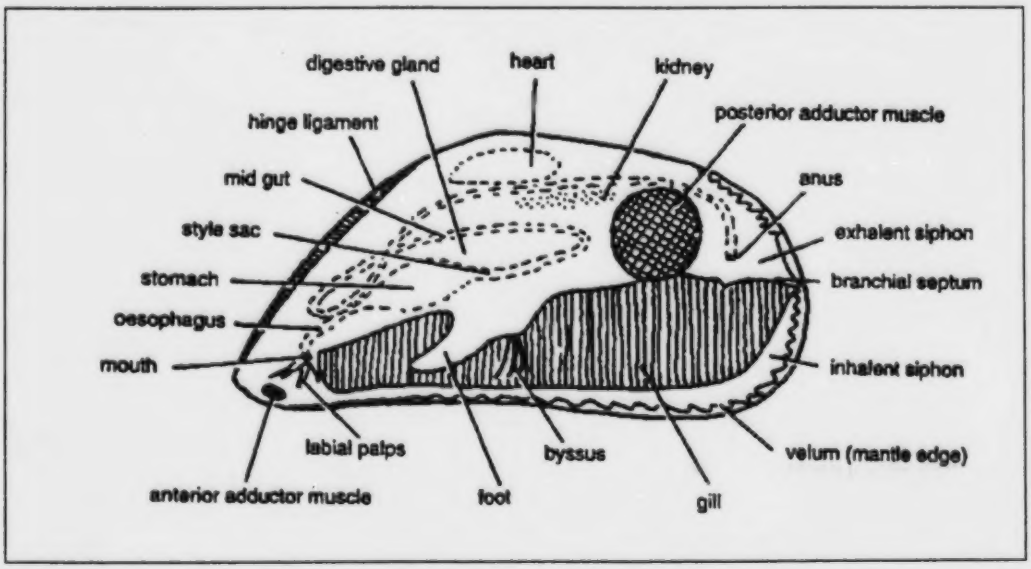

Figure 1: Diagram of the mussel showing the location of the principal organs. (from Scarratt, 1995)

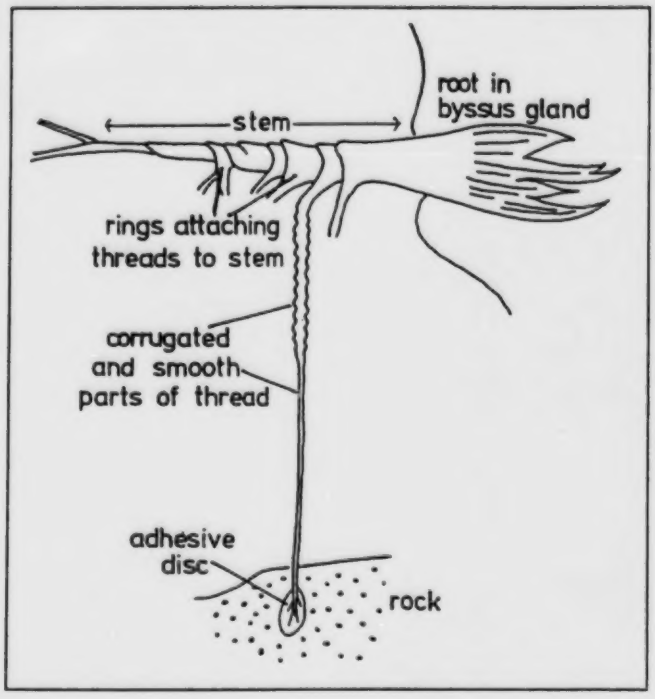

Figure 2: Morphology of the byssus. (from Smeathers & Vincent, 1979)

Depending on local conditions, the mussel may take three years to reach adult size, but can usually reproduce after one or two years. Reproduction is governed by the physiological state of the mussel, and is triggered by environmental conditions, such as temperature and food supply, which govern the induction of gonadal development. When the gonads are ripe, sperm and eggs are released; fertilization occurs externally in the surrounding seawater. In eastern Canada, spawning usually occurs in May-July, and within any year, multiple spawning periods are common (Scarratt, 1995).

LIFE CYCLE

When the proper set of conditions occur to initiate the spawning of sexually mature mussels, sperm and eggs are released in large quantities (up to $8x10^6$ eggs per individual, Bayne et al., 1978), for external fertilization. The actual quantity and quality of these gametes is determined to a large extent by the physiological condition of the adults. This in turn is governed by the level of stress (i.e. elevated temperature, starvation) the adults were exposed to prior to spawning. Generally speaking, the level of stress is directly related to the number and viability of the gametes released (Bayne et al., 1975; Bayne et al., 1978). Stress can also be introduced in the form of chemical contaminants. Kluytmans et al. (1988), studied the sublethal effects of cadmium on reproduction. They determined that exposure at the 100 ppb level, caused inhibition of follicle development in the gonads of both sexes and resulted in a lower number of gametes being produced and released. They were surprised to discover that cadmium (100ppb) also caused an increase in spawning frequency.

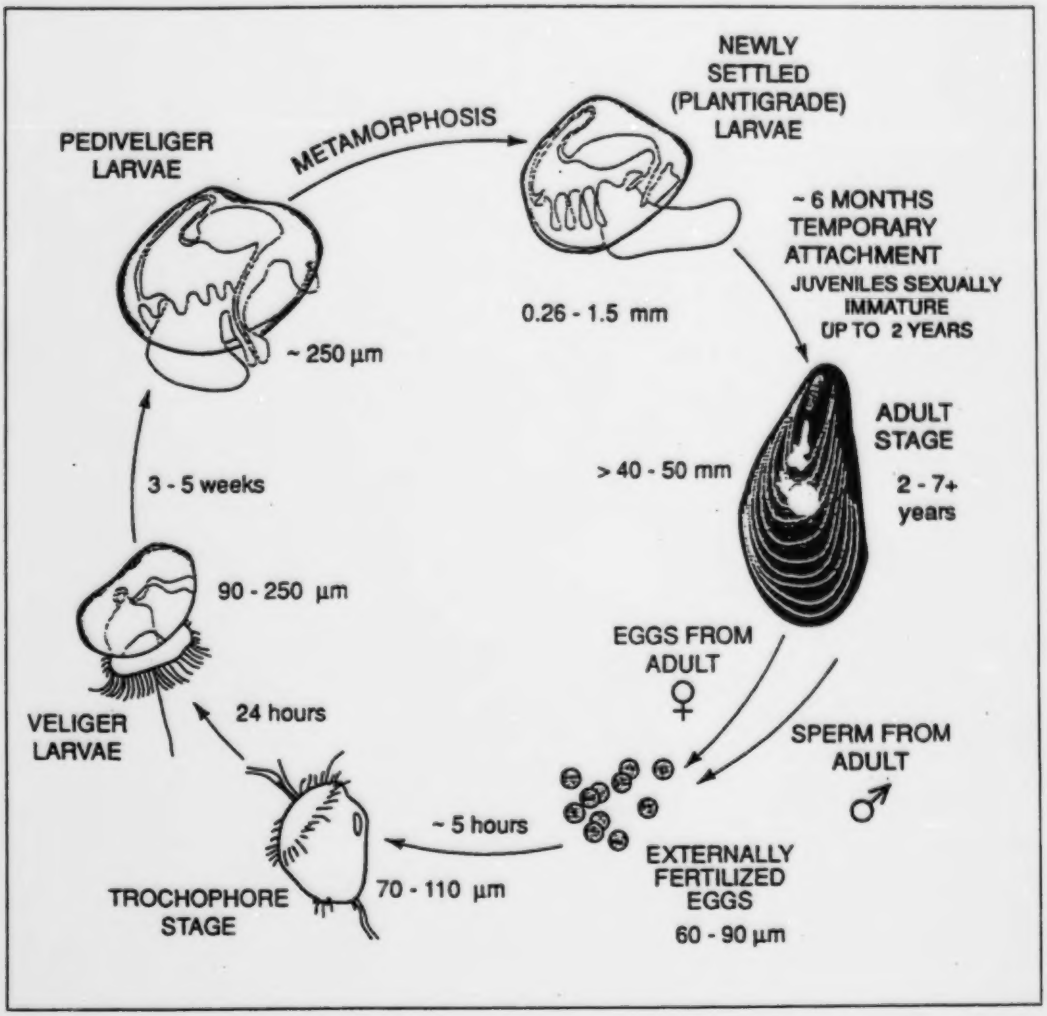

Figure 3: Generalized life cycle of blue mussel, *Mytilus edulis*, in eastern Canada. (from Stewart, 1994)

Once the gametes have been released, external fertilization takes place shortly. The fertilized eggs are 60-90 µm in size (Stewart, 1994). Four or five hours later(at 18°C) the embryo develops ci¹.a and begins to swim. At this stage, the developing embryo is referred to as a trochophore larvae. During the trochophore stage the larvae is generally 70-110 µm in size. The cilia are used for movement, but also function in the feeding of the trochophore. Following a further 24-48 hours, the trochophore develops a shell gland and begins to attain the initial larval shell. This stage is named for the resemblance (in shape) of the growing larvae to the letter D. Secretion of the second larval shell begins immediately. This is followed by the development of a ciliated velum (i.e. veliger larvae),

which permits swimming and feeding. Depending on conditions, the veligers range in size, from 90-220 μm.

During the next stage (3-5 weeks), the veliger grows quite rapidly and develops into a pediveliger, which has a foot (which allows larvae to crawl very effectively), eye spots and gills. The pediveligers are usually 250-300 μm in shell length, and are ready to begin site selection which occurs primarily by use of the foot. Site selection can be delayed weeks if a proper substrate cannot be located. The larvae seem to prefer rough or filamentous surfaces and agitation seems to encourage settlement (Eyster & Pechenik, 1987). At an appropriate site, the larvae secrete byssal threads and thereby anchor themselves to the substrate. If the site is unsuitable, the larvae are able to swim elsewhere. After settlement, pediveliger larvae metamorphose; the velum disappears and a reorientation of structures occurs. Organ systems increase in complexity and secretion of the adult shell begins (Widdows, 1991). At this post-larval stage, the newly settled mussels are called plantigrades.

FACTORS AFFECTING LARVAL DEVELOPMENT AND SURVIVAL

Prior to metamorphosis and the initial settlement of the pediveliger, several factors can affect success of the mussel. Assuming no unnatural or adverse conditions (i.e. pollution), the following natural variables seem to have the greatest effect on the development and survival of mussel larvae: (1) adequate food supply. Widdows (1991) determined a number of conditions that directly affect larval feeding. The concentration of food seemed to affect the rate at which mussel larvae could clear the water of food. The lower the concentration, the more effective the clearance rate. He also resolved that mussel larvae have maximum retention efficiencies for particles of 2-6 μm diameter and that particles that were >9 μm were not retained. Riisgard et al. (1980), had comparable results with mussel pediveliger retention efficiencies. Clearance rates were greatest for particles ranging 2.5-3.5 μm in size, while particles smaller than 1 μm or larger than 8-9 μm, could not be consumed. Widdows (1991) also determined that demand for food is directly related to larval size, with a maximum ingestion rate of 60-70% of larval body weight per day. From his experiments, he determined the maximum larval growth rate to be 12 μm day^{-1}. Bayne (1965) illustrated that larval growth was directly affected by the concentration of food. Both experimenters concluded that lack of an adequate food supply would delay growth, leading to an extended larval stage.

Perhaps the most important factor affecting larval development and survival is: (2) temperature. Widdows (1991) and Bayne (1965) found similar results for temperature effects. Mussel larvae reportedly have a maximum growth rate within 16-22°C, and the growth rate decreases outside this range. Bayne's results also indicated that feeding or clearance rates increased with temperature which would lead to an increased growth rate. Beaumont and Budd (1982) found that mussel larvae could be maintained at 5°C for 2

months with little growth, then at 17°C, growth resumed, followed by successful metamorphosis. They also found that the identical treatment of scallop (*Pecten maximus*) larvae caused complete mortality.

(3) Salinity and O_2 concentration play minor roles in the growth and development of mussel larvae. Provided the salinity has not been altered drastically, there seems to be very little effect. The O_2 concentration also appears to have limited effects on growth and development. Riisgard & Randlov (1981) concluded that O_2 consumption by pediveliger larvae was a function of size.

(4) Availability of an adequate substrate for settlement is the other important variable regarding growth/metamorphosis and survival. Through the use of a foot, pediveliger larvae seek adequate sites for permanent settlement. Site selection is based on the following criteria: hard, filamentous substrate; presence of other established mussels; and availability of food (i.e. adequate current). Widdows (1991) concluded, that the larval stage can range from 3 weeks to 3 months, and that metamorphosis is delayed if adequate settlement sites are not located. Such a delay can greatly affect survival of mussel larvae (Widdows, 1991; Bayne, 1965). Meanwhile, Beaumont & Budd (1982) and Pechenik et al. (1990), found that delaying metamorphosis had very little effect on mussel mortality.

During the metamorphosis phase there is a period during which the developing mussel is unable to feed. The velum disappears and the gills develop, but between the disappearance of the velum and the completion of the gill development, the mussel is incapable of food acquisition, resulting in zero growth. However, Lane et al.(1982, 1985) discovered that pediveliger larvae develop a gland near the foot for a very specialized function. This gland produces a structure similar, but very distinct from the byssal thread. This 'drifting thread' is utilized during site selection for mobility. After the pediveliger has found a site, but decides to relocate, it can break the byssal attachment to re-enter the water column. The pediveliger then propagates a 'drifting thread' which aids in this relocation. Lane et al. (1985) determined that young post-larval mussels (up to 2mm shell length) use this thread to aid in floatation in the water column, resulting in a sinking rate of 0.1 cm/s. Resettlement continues (sometimes for months) until the plantigrade mussel has found what appears to be an adequate site for long-term survival. The mussel is now termed a juvenile, and continues to grow and mature. Sexual maturity usually occurs after 1-2 years of age. The mussel life cycle is then complete.

CONTROLLING MUSSEL ACCUMULATION - ANTIFOULING

INTRODUCTION

The problem of marine fouling is timeless. In the days of wooden ships, deterioration due to marine organisms was visually evident. In order to protect the integrity of the hull, the practice of sheathing became popular. Prior to the 18th century, lead was the material of choice for sheathing. However, the 18th century saw a rise in the popularity of copper sheathing (Field, 1981). That's when it was discovered that the dissolution of copper provided the protection (Anon., 1978a). When ship construction started utilizing metals for hulls, copper sheathing caused a corrosion problem, so copper sheathing was abandoned.

The use of the oceans has steadily increased. Today the ocean is still heavily used for cooling and energy extraction. Technology and modernization have improved the efficiency of ships and navigation, but have provided new avenues for the accumulation of marine life (biofouling). In particular, biofouling restricts flow in cooling water systems and decreases the efficiency of heat exchangers. Extensive/uncontrolled biofouling eventually leads to serious equipment failures, and the potential for great financial losses.

One solution to this biofouling problem was to control the accumulation by way of toxic substances. The most commonly used substance during the past couple of decades has been chlorine in several different forms (White, 1972; Claudi & Mackie, 1994b). Chlorine, however, not only has detrimental effects on the biofouling organism, but it also has deleterious effects on non-target organisms (see section 3.3.a). As a result of increased environmental awareness and in an effort to find economical, efficient solutions to biofouling, research is now focused on finding methods which discourage or repel the settlement of biofouling organisms, rather than causing mortality (Callow et al., 1986; Clare, 1998).

This report will review both existing methods of antifouling, and those that are currently being developed (Table 1). Due to the biofouling problem in the cooling systems of ships, this review will naturally focus on the applicability of antifouling techniques to marine cooling water systems. The parameters of the cited examples and methods will be reported where possible and each method will be discussed in terms of: (1) effectiveness as an antifoulant, (2) applicability, (3) hazards, and (4) costs. All such details were not always available, for the emerging methods. A brief discussion on biofilms will also be included to examine their interaction with macrofoulers (i.e. mussels).

Table 1 contains known and developing methods of antifouling, organized into two main categories, chemical and nonchemical antifoulants. The chemical antifoulants consist of oxidizing and nonoxidizing agents. The category of nonchemical antifoulants consists of: (1) thermal related treatments, (2) nonthermal energy (i.e. acoustic, electrical, electrochemical, gamma or UV radiation, and ultrasonic vibration), (3) hydraulic (i.e. water jet cleaning, water velocity), (4) mechanical (i.e. Amertap, American M.A.N., pipe robot), (5) miscellaneous (i.e. anoxic water, low energy coatings, osmotic shock, etc.). For the products that were found on the Internet, an address will be provided where possible.

CHEMICAL ANTIFOULANTS: OXIDIZING AGENTS

CHLORINE (Table 2)

Chlorination was used for the control of marine growth in North America, beginning in 1929, and the first reported control of a mussel infestation was in 1949 (White, 1972). In North America, chlorination for the purpose of disinfection, has occurred since 1892 for wastewater, and since 1908 for drinking water (Van Benschoten et al., 1993). Chlorine is the most common disinfectant and biofouling control agent in use in North America.

Chlorine, a strong oxidizing agent, is available in several different forms. Chlorine (Cl_2) is a greenish-yellow gas, which is added directly to a stream of water. Sodium hypochlorite (NaOCl) is a liquid that can be obtained at various concentrations. The reactive species in water (hypochlorous acid, HOCl) is the same for both Cl_2 and NaOCl. (See the equations below.)

$$Cl_2 + H_2O \longleftrightarrow HClO + HCl$$

$$NaOCl + H_2O \longleftrightarrow HClO + NaOH$$

Depending on pH and to a smaller extent temperature, the hypochlorous acid could further dissociate to give:

$$HOCl \longleftrightarrow OCl^- + H^+$$

Further reactions between compounds dissolved in seawater and chlorine will be discussed later. It should be mentioned that chlorine can also be generated by electrolysis

Table 1: Possible Methods of Controlling Mussel Infestation

Chemical Antifoulants	Nonchemical Antifoulants
Oxidizing Agents Bromine Bromine Chloride Chlorine Chlorine Dioxide Hydrogen Peroxide Hydrogen Peroxide + Fe ion Iodine Ozone Potassium Ferrate Potassium Permanganate	Thermal Heat Shock Freezing Nonthermal Energy Acoustic Energy Electrical Electrochemical Gamma Irradiation Ultraviolet Radiation Ultrasonic Vibration
Nonoxidizing Agents Ammonia and Amines Carbamate Compounds Cyano Compounds Metals (Salts) Molecular Regulators Natural Extracts and Analogs Organometals Other Paint Additives	Hydraulic Water Jet Cleaning Water Velocity Mechanical Amertap American M.A.N. Pipe Robot
	Miscellaneous Anoxic Water Low Energy Coatings New Paint Formulations Osmotic Shock Combinations Internet Products Biofilm

of seawater. To do so, a current is passed through seawater. Chlorine (Cl_2) gas is generated at the anode, while hydrogen (H_2) gas is produced at the cathode. Once the chlorine gas comes in contact with water, the above reactions occur, producing the same reactants (i.e. OCl^- and H^+).

The oxidizing effects of chlorine as a biofouling agent are well documented. Over the years, theories have developed to explain this action, but the mechanism is still not well understood. The current theory postulates that oxidation occurs following diffusion through the cell wall (Claudi & Mackie, 1994b). The effects of chlorine seem to concentrate on certain tissues in marine organisms, but further research is required to fully understand the mode of action.

Regardless of the choice of chemical agent, the treatment can be administered in 5 ways: (1) end-of-season treatment, (2) periodic treatment, (3) intermittent treatment, (4) semi-continuous treatment, and finally (5) continuous treatment (Claudi & Mackie, 1994a,b).

For the end-of-season approach, an antifouling agent is added for a period of time that is sufficient to kill all the established adults in the system. This treatment is usually timed to coincide with the end of the breeding season, when adult mussels have minimal energy reserves and tend to be most susceptible.

The second treatment, periodic treatment, is essentially a more frequent application of the end-of-season option. Again, adult mussels are the target. The periodic and end-of-season treatments are considered reactive methods because application occurs after the mussels are established.

The following three treatments are considered proactive because the goal is prevention of larval settlement. Intermittent treatment involves dosing at frequent intervals (i.e. every 6 or 12 hours) in an attempt to prevent settlement and infestation. The target in this case is mussel larvae, which are less resistant than the adults, and therefore require a lower chemical concentration and duration of application, to achieve results. However such treatment is ineffective against established adult mussels (Claudi & Mackie, 1994a).

Semi-continuous treatment was developed after the response of mussels to many chemical irritants, including oxidizing agents and particular metals was discovered. When exposed to certain substances, mussels stop filtering (in most cases) and quickly close their valves (shells), thereby preventing further exposure to the irritant. Dosing intervals can be determined with reference to the time the mussels remain closed. Eventually, due to metabolic demands, the mussels begin filtering again, and become further exposed to the antifouling agent.

The last option, continuous treatment, is designed to discourage any settlement in the system. Although incoming larvae may not suffer 100% mortality, the presence of the

antifouling agent may be adequate to discourage settlement. Low level chemical addition, if carried out over the entire breeding season, will cause any established mussels to succumb, or detach and attempt to leave the treated area. The concentration of the antifoulant required can be quite low, but the application must be continuous.

Another consideration when contemplating chlorine treatment, concerns chlorine demand. Due to competing reactions in seawater, not all the chlorine added to the system will react with water to yield hypochlorous acid. The additional chlorine required to achieve the necessary hypochlorous acid concentration in the system, is dependent on the chemical content of the seawater and the flow-through time of the system. This additional demand varies depending on local water composition. That is, demand in a marine port will probably exceed the demand in the open ocean, due to a higher chemical content in port seawater.

The experimental results from the literature are summarized in Table 2. The temperature dependency of chlorine efficacy, as an antifoulant, is well known. Practically all the literature acknowledges the increasing effectiveness of chlorine at elevated temperatures. This implies that at lower temperatures, a higher chlorine concentration is required for adequate control. It is uncertain whether it is due to reduced oxidizing capacity of chlorine, or reduced metabolic rate in mussels at lower temperatures.

Chlorination is used for the control of many different fouling organisms, such as to control a thin layer of the slime-forming bacteria (biofilm) in heat exchangers, that will cause a significant decrease in efficiency. Both Panchal et al. (1984) and Cole (1977) determined that intermittent treatment was adequate to control the biofilm problem. Panchal et al. (1984) calculated the adequate application method for a system with aluminium piping and a flow rate of 4-6 ft/s, was to maintain a chlorine (produced by electrolysis of seawater) concentration of 70 ppb (parts per billion ~ µg/L) for 1 hr/day. This procedure provided sufficient control of the biofilm for about 700 days.

Several authors (Burton & Liden, 1978; Burton & Margrey, 1979; Mayack et al., 1984) considered chlorination for controlling all forms of biofouling. They found that intermittent dosing was inadequate for treating all forms. Mayack et al. (1984) used sodium hypochlorite with a reduced flow rate of 2 cm/s and determined the following: that continuous dosing at 0.15 ppm (parts per million ~ mg/L) was more effective than either 1 ppm for 1 hour/day, or 1 ppm for 15 minutes administered four times a day. The intermittent treatment did not seem appropriate for the more resistant organisms (like mussels).

White (1972) examined the effects of chlorination on *Mytilus edulis* and found that a continuous application at 0.25-0.5 ppm (above 40°F) was adequate for a flow-through system. Rajagopal et al (1995c) studied the effects of chlorine at various (0.25-1.5 ppm) concentrations on the green mussel, *Perna viridis*. They found that smaller mussels were less tolerant than larger mussels, and that the green mussel was able to detect (by valve closure) chlorine at the 0.25 ppm level in seawater. They observed that

Table 2: Oxidizing Agents- Chlorine (Cl_2, HOCl, etc)

Species	Life Stage	Temperature	Duration	Concentration	Results	Notes	Author
Asiatic clam	Juvenile	7° C	672 hrs	0.29 mg TRC[1]/ L	39% mortality	Freshwater	Belanger et al (1991)
(*Corbicula fluminea*)	Adult	7° C	672 hrs	0.29 mg TRC[1]/ L	3% mortality	Flow-through system	
	Juvenile	23° C	408 hrs	0.29 mg TRC[1]/ L	100% mortality		
	Adult	23° C	672 hrs	0.29 mg TRC[1]/ L	67% mortality		
Asiatic clam		10-17° C	1 hr/day	1 mg/L	60% less control	Freshwater	Mayack et al (1984)
(*Corbicula fluminea*)		10-17° C	15 min, 4x/d	1 mg/L	50% less control	Flow-through system	
		10-17° C	continuous	0.15 mg/L	55% less control		
Marine mussel	Adult	Summer	4h on/4h off	0.2 mg FAH[2]/ L	growth rate halved	Seawater	Jenner (1983)
(*Mytilus edulis*)	Adult	Summer	continuous	0.2 mg FAH[2]/ L	reduced growth	Power plant exper.	
Marine mussel	Adult	Warm	96 hrs	10 mg TRC/ L	100% mortality	Seawater, continuous	Claudi & Mackie (1994b)
(*Mytilus*)			120 hrs	2.5 mg TRC/ L	100% mortality	chlorination.	
			360 hrs	1.0 mg TRC/ L	100% mortality		
Biofilm		Warm	1h/day for 700 d	0.7 mg/L	controlled biofouling	Cl_2 by electrolysis	Panchal (1984)
Green mussel	Adult (12mm)	29° C	504 hrs	1 mg/L	100% mortality	Seawater	Rajagopal et al (1995c)
(*Perna viridis*)	Adult (95mm)	29° C	816 hrs	1 mg/L	100% mortality	Flow-through system	
	Adult (12mm)	29° C	30 hrs	10 mg/L	100% mortality		
	Adult (95mm)	29° C	48 hrs	10 mg/L	100% mortality		
Zebra mussel	Adult (2-10mm)	12° C	360 hrs	1 mg/L	50% mortality	Freshwater	Martin et al (1993a)
(*D. polymorpha*)		12° C	240 hrs	2.5 mg/L	50% mortality	Static	
		12° C	144 hrs	5 mg/L	50% mortality		
		12° C	96 hrs	8 mg/L	50% mortality		
		12° C	72 hrs	5 mg/L	14% mortality		
		12° C	96 hrs	5 mg/L	43% mortality		
		12° C	120 hrs	5 mg/L	45% mortality		
Zebra mussel	Adult (2-8mm)	22° C	295 hrs	1 mg/L	50% mortality	Freshwater	Martin et al (1993b)
(*D. polymorpha*)		22° C	178 hrs	2.5 mg/L	50% mortality	Static	
		22° C	157 hrs	5 mg/L	50% mortality		
Zebra mussel	Larvae	16-26°C	~27 min	1 mg/L	30% mortality	Freshwater, flow-through	Klerks et al (1993)
(*D. polymorpha*)	(veligers)						
Marine fouling	various	May-Oct	15min/3hr	0.2 mg TRH[3] /L	Continuous worked	Seawater, flow-thru,	Burton & Margrey (1979)
				0.5 mg TRH/L	better than intermit.,	field test at power	
			continuous	0.1 mg TRH/L	especially during high	plant.	
				0.3 mg TRH/L	fouling season.		
Marine fouling(soft)	various	> 40°F	3times/day	1.0 mg TRC/L	Control level	Seawater, flow-thru	White (1972)
Marine fouling(hard)		> 40°F	continuous	0.25-0.5 mg TRC/L	Control level	(general review)	
Marine fouling	various	25° C	continuous	1.0 mg TRC/L	Complete fouling control	Electrolysis of Seawater, flow-thru	Nayar & Ragunathan (1989)

TRC[1]- Total Residual Cl
FAH[2]- Free Available Halogen
TRH[3]- Total Residual Halogen

filtration rate, foot activity and byssal thread production declined as chlorine concentration increased. Rajagopal et al.(1995c) concluded that intermittent chlorine dosing was inadequate for controlling mussel infestation. Continuous, low-dose chlorination (i.e. 0.2 ppm) was effective, but did not cause mortality. The authors hypothesized that settlement was deterred due to negative effects on metabolism.

It is apparent that chlorine is an effective antifouling agent when the mussel is the target organism. However, chlorine treatment has received much attention recently due to related environmental effects. Chlorine is non-selective and its application can result in many negative consequences on non-target organisms (Brungs, 1977; Bender et al., 1977). In addition, chlorine also tends to react with organic compounds in seawater, and results in the formation of chlorinated methane compounds (CH_3Cl, CH_2Cl_2, $CHCl_3$), some of which are known carcinogens. For these reasons, the use of chlorine has been heavily regulated by environmental agencies (EPA), resulting in an ever-decreasing, allowable level of residual chlorine release to the environment. Present allowable levels may already be below effective concentrations necessary for satisfactory biofouling control in once-through cooling systems. If this is the case, dechlorination would be necessary before discarding the treated cooling water.

Other factors to be considered before choosing chlorine treatment are: cost, ease of handling, adaptability and effect on the system. The cost of chlorine remains very reasonable in comparison to alternatives, although if dechlorination is required, it will add significantly to the overall cost. Chlorine gas is a very toxic substance and is marketed in pressurized, gas cylinders, making the handling of the product very dangerous. For this reason, sodium hypochlorite is the obvious choice since it can be obtained in directly useable liquid form. Electrochemical generation of chlorine from seawater would be a better alternative in terms of handling, but costs are difficult to determine. Space limitations are also a concern for all the applications. Gas cylinders require special care for on-site storage. The sheer volume of sodium hypochlorite necessary for continuous application could make on-site storage impossible. The equipment essential for the treatment of large volumes of water by electrolysis also requires a large space.

In order to use chlorine as an antifouling agent, cooling water systems would require adaptations. If chlorine introduction into the system was in the form of gas or liquid, an appropriate injection apparatus would have to be retrofitted for the system. Ideally, these injectors would be located at all cooling water intakes. Otherwise, an electrolysis apparatus would have to be placed internally into the system at all the intakes. Depending on the number of cooling water intakes, this could prove to be very expensive.

Another consideration prior to the selection of an antifouling agent is system effect. A method that effectively controls the fouling problem, but seriously compromises the cooling system integrity is of little value. Therefore consideration must be given to the composition of the cooling water system. The concern in this case, is the corrosion of the metals used in the cooling water system. White (1972) determined that the continuous

addition of 1 ppm chlorine had no adverse corrosion behavior on steel and many copper-base alloys.

Chlorine has been, and to a large extent, still is the cornerstone of the disinfection industry. In this report the remainder of the oxidizing agents will be evaluated in comparison with chlorine. If and when actual numerical data is available, that will also be presented. Comparisons with nonoxidizing chemicals and nonchemical methods will be presented when available.

CHLORAMINE (Table 3)

When the product of chlorine and water comes in contact with nitrogen-containing compounds, a reaction occurs to form one of the chloramines:

$$HOCl + NH_3 \longleftrightarrow NH_2Cl \text{ (monochloramine)} + H_2O$$

This reaction is pH dependent, so at low pH, the prevalent species is $NHCl_2$ (dichloramine), while at elevated pH, NH_2Cl is the main species present (Claudi & Mackie, 1994b).

The primary use of chloramines has been the disinfection of drinking water. Chloramines are generally considered to be less powerful oxidants than hypochlorous acid. For this reason there has not been a great deal of research into chloramines as biofouling control agents. In a freshwater study (Belanger et al., 1991), on the Asiatic clam (*Corbicula fluminea*) at 20°C, monochloramine had a 48-h LC_{50} of 0.078 mg/L. This is the concentration at which 50% mortality was achieved in 48 hours. There was no data available on a seawater application.

Chloramines have several advantages over chlorine. First, chloramines are available in a ready-to-use, liquid form that does not require any special storage facilities, and they are less of an environmental concern because they do not tend to form chlorinated methane compounds, like chlorine. The cost in comparison to chlorine was not available, but one can assume that costs would probably be similar to those for chlorine.

BROMINE (Table 3)

The primary use of bromine has been in the treatment of swimming pool water. It is very similar to chlorine when introduced to water, producing a very comparable oxidizing agent.

$$Br_2 + H_2O \longleftrightarrow HOBr \text{ (hydrobromous acid)} + HBr$$

The information available on the use of bromine in controlling biofouling is very limited. It has been used successfully as a biocide in a few small-scale freshwater applications.

The antifouling properties of bromine are similar to chlorine in both action and effectiveness. Actually, at higher pH (9.0)values, bromine is more effective than chlorine, although it is less effective at lower pH (6.0) values (Waite & Fagan, 1980; Claudi & Mackie, 1994a).

Since the water chemistry is very similar to that of chlorine, reactions with organic compounds in seawater, forming brominated organic compounds, are likely. The literature available on this topic is very limited in comparison with the chlorinated compounds. There is some indication that the brominated organics are more stable (therefore more of a concern) in seawater than their chlorinated counterparts (Waite & Fagan, 1980).

Liquid bromine is highly corrosive, making shipping and handling difficult. The availability of bromine on a large-scale would probably prove troublesome, not to mention expensive. Bromine would be several times more expensive than chlorine, therefore would not be considered a logical alternative to chlorine (Burton & Liden, 1978; Waite & Fagan, 1980).

BROMAMINE

Like chlorine, hydrobromous acid will react with nitrogen containing compounds to give a similar product. Like chloramines, bromamines are not as powerful an oxidizing agent as chlorine or bromine. The other properties of chloramines would essentially apply to bromamines. Although it is believed that bromamines are superior to chloramines as biofouling control agents and less harmful to the environment (Mills, 1980; Burton & Margrey, 1979; Waite & Fagan, 1980). This work was also carried out in freshwater, and little knowledge regarding effectiveness and byproducts, in seawater, was available.

BROMOCIDE (Table 3)

This is a commercial term that refers to those compounds that release bromine to control biofouling. A wide range of these compounds are available. They behave essentially in the same manner in water as bromine. Most are available as a solid (usually pellet) or liquid form, and lack the highly corrosive nature of liquid bromine.

One compound, a mixture of brominated phenols, was found to be effective in controlling the freshwater clam (*Corbicula fluminea*) at fairly low concentrations. The 48-h LC_{50} was 0.092 mg/L. That is, the bromocide concentration required to kill half the clams in 48 hours was 0.092 mg/L. This was not as effective as a chlorine compound tested at the same time, which had a 48-h LC_{50} of 0.078 mg/L (Belanger et al., 1991).

Brominated compounds generally are easy to handle and administer, but they are usually costly and untested in seawater in comparison to their chlorine counterpart. Because of this their impact on the environment is unknown. Normally, for freshwater applications, they are not recommended for once-through systems. Often some treatment (to eliminate residual bromine) is required before they can be released to the environment. For these reasons, brominated compounds are not likely alternatives to chlorine treatment.

BROMINE CHLORIDE (Table 3)

This oxidizing agent is formed by mixing equal portions of bromine and chlorine. The result is a gas that is stored in pressurized gas cylinders, much the same as chlorine. Reaction with water produces the same oxidizing compound as bromine, which is hypobromous acid (HOBr). Therefore, it should be very similar to bromine in mechanism of action and effectiveness. It has advantages over bromine in that it is more water soluble and less corrosive.

Most studies of bromine chloride have been in fresh or low-salinity water. In this environment, several researchers have found this compound to be as effective as chlorine at a comparable concentration (Mills, 1980; Wackenhuth & Levine, 1977; Burton & Margrey, 1979). All the studies indicated that continuous application controlled biofouling better than intermittent applications.

In the presence of nitrogen compounds, the same reaction occurs with bromine chloride, as would occur with bromine or chlorine. That is, the formation of bromamines and/or chloramines. If the nitrogen level is high in the water to be treated, the required concentration of bromine chloride can be affected. In any case, more seawater related work is necessary, in order to truly evaluate bromine chloride as an alternative to chlorine.

Bromine chloride is a gas like bromine and chlorine, therefore the handling and storage requirements would be similar. The equipment necessary for application is identical to that used for chlorine injection. The two drawbacks of bromine chloride are supply and cost. It may be difficult to secure a reliable supply of BrCl in large quantity. Even if that were possible, the cost would still be several times greater than that of chlorine.

A product, similar to bromine chloride, was found during an Internet search. The product, called M-725 (website: http://www.magnacheminc.com/products/m725.html) is a mixture of dichlorodimethylhydantoin and bromochlorodimethylhydantoin. The manufacturer makes many claims about it's effectiveness, but did not provide any applications data. This product is available in a briquette form which is very soluble and the mode of action apparently stems from the halogenated amines.

CHLORINE DIOXIDE (Table 3)

Chlorine dioxide (ClO_2) is a corrosive, reddish-yellow gas with oxidizing powers that are 2.5 times that of chlorine. It is very soluble in water, quite reactive and sufficiently unstable that it has to be generated on site. The distinct advantage that chlorine dioxide has over chlorine is it does not react with nitrogen compounds to form chloramines. It also does not react with organic compounds to form any of the chloromethane compounds, which are of concern.

The only sources of information about the application and effectiveness of chlorine dioxide as an antifoulant, were related to freshwater. There seems to be a complete lack of seawater related data. Adult zebra mussels (15-20mm) in a flow-through tank, continuously exposed to 1 ppm chlorine dioxide, showed 100% mortality in 8 days (Khalanski, 1993). The author also noted that fish exposed to much lower levels of chlorine dioxide, died within 24 hours. Another study by Matisoff et al. (1991), produced a LC_{50} of 0.4 ppm for zebra mussel mortality. Van Benschoten et al. (1993), determined that zebra mussel larvae were more resistant to chlorine dioxide than chlorine or chloramine. All these studies were carried out on the freshwater zebra mussel (*Dreissena polymorpha*). Several authors (Mayack et al., 1984; Claudi & Mackie, 1994a; Waite & Fagan, 1980) have indicated that chlorine dioxide is more potent than chlorine in controlling many forms of freshwater bacteria.

Chlorine dioxide seems like a good alternative to chlorine in freshwater. Due to the lack of information on saltwater effectiveness, however, it is impossible to recommend it as a reasonable alternative to chlorination. Considering the inconvenience and hazard of on-site production, space requirements, cost, and possible environmental concerns, it is not a promising alternative.

HYDROGEN PEROXIDE (Table 3)

Hydrogen peroxide is a naturally occurring compound found in rain and seawater. It has a high oxidizing potential, similar to chlorine, but unlike other strong oxidizing agents, is not a good disinfectant. It can be obtained at various concentrations in the liquid form, making handling, storage and application, simple and safe. Unfortunately, to control biofouling, hydrogen peroxide is required at relatively high concentrations, with long contact times.

One study (Van Benschoten et al., 1993) performed on zebra mussel (*Dreissena polymorpha*) larvae in a freshwater flow-through system, at 22°C, required 9.0 mg/L to achieve 95% mortality. The hydrogen peroxide was applied semi-continuously, dosing for 30 minutes every 12 hours. Another study (Martin et al., 1993a) using adult zebra mussels (2-10mm), was conducted under static freshwater conditions, and investigated the effects of hydrogen peroxide treatment at two different temperatures and several concentrations. At 22°C, Martin et al. determined that a 100% mortality rate, at concentrations of 30, 20 and 12 mg/L, required 72, 120 and 408 hours, respectively. At 12°C, the same experiment produced a 100% mortality for hydrogen peroxide concentrations of 30 mg/L and 20 mg/L, in 576 and 648 hours, respectively. Effectiveness of hydrogen peroxide is temperature-dependent.

The literature on seawater applications of hydrogen peroxide, included two studies that were done in flowing seawater for a 90-day duration. The first study (Nishimura et al., 1988) involved inhibiting the settlement of mussel (*Mytilus galloprovincialis*) larvae. The results indicated that a concentration of 1 ppm or more was required to achieve 90% settlement inhibition compared with the control. The second study (Ikuta et al, 1988) was done in the field (average temp. 19.4°C), so the native mussels (Japan) were utilized. Interestingly, the results implied that mussel fouling increased at lower (0.25-0.5 ppm) concentrations of hydrogen peroxide, and decreased at the higher concentrations of 0.5- 4.0 ppm.

Based on these results, it is obvious that hydrogen peroxide is not as effective as chlorine (based on concentration), at controlling mussel fouling. Although it has certain advantages over chlorine, such as safety and handling concerns, larger volumes would be required for adequate control, dictating greater space demands. The larger volume required for effectiveness would also lead to higher costs. For this reason one author (Claudi & Mackie, 1994a) stated that hydrogen peroxide would not be recommended for flow-through systems, due to cost alone.

HYDROGEN PEROXIDE + IRON (Table 3)

This is a combination of the oxidizing agent (H_2O_2) and in most cases, ferrous sulphate ($FeSO_4 \cdot 7H_2O$). As previously mentioned, the environmental concerns attributed to hydrogen peroxide are believed to be very few. The same would apply to ferrous sulphate. As such, this combination has received considerable attention. The present theory implies the iron ion helps catalyze the oxidation reaction, however, no experimental proof has been presented to date.

The literature contained several studies that utilized the combination in attempts to control biofouling. Two studies (Klerks et al., 1993; Klerks & Fraleigh, 1991) on the freshwater zebra mussel (*Dreissena polymorpha*), at different life stages, utilized Lake Erie as a natural water source for an experimental flow-through system. The exposure of mussel veligers to 1.0 mg/L peroxide and 0.25 mg/L iron for 27 minutes, resulted in a mortality rate of only 7% (Klerks et al., 1993). A previous study using adult zebra mussels (14-16mm) in a similar flow-through apparatus, with a temperature range of 17-27°C, produced comparable results. An 8 week exposure to 5 mg/L peroxide and 1.25 mg/L iron concluded with a 30% mortality of the adult mussels (Klerks & Fraleigh, 1991). Obviously, this is not an effective combination for controlling freshwater mussel fouling.

Seawater results were somewhat different, two Japanese studies (Nishimura et al., 1988; Ikuta et al., 1988) were performed in the field, using natural seawater in a flow-through system with a reduced flow rate (0.1 m/s) and continuous application (for 90 days). The first examined the inhibition of larval settlement in the presence of the peroxide/iron antifoulant at different concentrations. Results indicated that 0.25 mg/L of both chemicals was sufficient to inhibit settlement of 90% or more of the fouling organisms (Nishimura et al., 1988). The second study involved the control of macrofouling organisms by application of various concentrations of the two chemicals. When hydrogen peroxide (1 ppm) was combined with iron (0.1-0.16 ppm), it produced almost a 95% reduction in fouling organisms compared to the control (Ikuta et al., 1988).

The results from seawater experiments are encouraging with respect to using hydrogen peroxide and ferrous sulphate to control biofouling. Further studies would be necessary to confirm that the combination does not pose any deleterious effects on equipment or the environment. The combination is not as cost-effective or capable an antifoulant as chlorine, but considering the environmental reputation of chlorine, it could be considered a possible alternative. Both hydrogen peroxide and ferrous sulphate can be handled and stored with a minimum concern for health and safety.

IODINE (Table 3)

Iodine, the least abundant of the halogens (i.e. Br, Cl) is a blue-black solid which has the lowest water solubility. Traditionally, it has been used for treating swimming pool and potable waters. As a halide, it undergoes the identical reaction with water as bromine and chlorine, giving hypoiodous acid (HOI).

Iodine has proven to be capable against freshwater bacteria in small-scale applications. No data is available on its use as a biofouling control agent in fresh or saltwater. It has some distinct advantages over chlorine in that it does not appear to react readily with nitrogen or organic compounds, making it a better choice environmentally. The problem with considering iodine as an alternative to chlorine is the availability and cost. It is the most costly of the halides considered.

OZONE (Table 3)

Ozone (O_3) is an extremely strong oxidizing agent and under atmospheric conditions exists as an unstable, pale blue gas. Other than chlorine, it is the oxidant which has aroused the most interest as a disinfectant in large-scale applications. Ozone persists for a much shorter time than chlorine in water treatment applications. From an environmental standpoint, this is an advantage over chlorine, however, from a treatment perspective (especially if the transit time through the system is relatively long), this is a disadvantage. Since ozone is such a strong oxidant, it shares a problem with chlorine in marine applications: the formation of halogenated organic compounds, through the oxidation of bromine present in seawater (Waite & Fagan, 1980).

Many studies have been conducted on ozone over the years. There are a number of ozone applications in freshwater. One report concluded, that as a bactericide and virucide in suspended systems, ozone worked as well as chlorine (Waite & Fagan, 1980). Other freshwater studies were performed on the zebra mussel (*Dreissena polymorpha*) at various life stages. Claudi & Mackie (1994a), reported that a 100% mortality of veligers and post veligers in the water column, when exposed to ozone at 0.5 mg/L (15-20°C), required a minimum contact time of 5 hours. This same source reported 100% mortality of adult mussels if exposed to 0.5 mg/L (or greater) for 7 to 12 days. The authors concluded that time to death depended on both concentration of ozone and water temperature. Van Benschoten et al. (1993), reported that zebra mussel veligers, exposed to ozone (0.5 mg/L) in a flow-through (30 min. residence time) system, showed a 99% mortality at 16°C. The same authors reported a 100% mortality of adult (16mm) zebra mussels exposed to 1.1 mg/L of ozone, at 20°C, for 14 days. Ozone seemed to be as effective as chlorine at controlling zebra mussel infestation, but there was a relatively long lag time between application and mortality. Also, ozone seemed to destroy mussel byssal threads.

The published data concerning ozone treatment of marine mussels (*Mytilus edulis*), included an investigation of effects of ozone exposure on the larvae (3 and 20 days old) (Toner & Brooks, 1977). Ozone exposure was performed for a range of concentrations and three different temperatures. The exposure consisted of a 5 minute treatment with ozone, after which, the larvae were left in the ozonated water for 24 hours. For both larval stages tested, the results were identical when average % of survival was used to assess the data. Survival of larvae decreased as temperature increased, although the effect was more pronounced for the younger larvae. The authors concluded that ozone would only be effective against relatively small larvae, provided continuous application was performed. A review article reported that an industrial (condenser system) application of ozone treatment in seawater, proved no better than chlorine, and under certain conditions appeared to be much less effective than chlorine, at controlling biofouling (Waite & Fagan, 1980).

From the information available, it would be difficult to endorse ozone as an adequate replacement for chlorine treatment. Ozone is extremely volatile, requiring on-site production through special equipment. Since ozone breaks down so quickly, several points of injection would be necessary for adequate control of biofouling in a large cooling system. Costs alone could quickly eliminate ozone as a possible alternative. Some concern exists about the potential for accelerated pipe corrosion due to ozone treatment.

POTASSIUM FERRATE (Table 3)

Potassium ferrate (K_2FeO_4) is a blackish, purple crystal, that turns water a deep purple colour. The ferrate ion (Fe^{6+}) is a strong oxidant, that is produced electrochemically and does not occur in nature. It is a fairly stable solid, which makes handling and storage, convenient and safe.

Application of potassium ferrate(~ 2 mg/L, twice daily), proved very effective at inhibiting biofilm formation, in one freshwater study (Waite & Fagan, 1980). Potassium ferrate treatment provides adequate disinfection of water and wastewater, unfortunately, there was a complete lack of information on marine applications.

Environmentally, potassium ferrate should not pose a toxicity problem. It does not appear to be a strong enough oxidant to oxidize chlorine, although it will oxidize bromine at very low pH and when bromine is present in high concentration. Supply and costs could certainly be a problem when large quantities are needed. Since so little information is available on potassium ferrate, much more research is required before it could be considered as an alternative to chlorine treatment.

Table 3: Oxidizing Agents- Excluding Chlorine

Compound Tested	Species Tested	Evaluation	Notes	Author
Bromine	aquatic organisms	As effective as Cl_2 on small-scale unproven on large-scale	Review article	Waite & Fagan (1980)
Bromocide (mixture brominated phenols)	Asiatic clam (*C. flumines*)	Found to be less effective than Cl	Freshwater tested at 30° C	Belanger et al (1981)
Bromine Chloride	Marine fouling	As effective as Cl_2, Br_2 on small-scale	Review article	Waite & Fagan (1980)
	Marine fouling	As effective as Cl_2 on weight basis tested at power station	Review article	Mills (1980)
	Marine fouling	As effective as Cl_2, but required larger quantity of BrCl, tested at electric station.	Seawater cooling system in summer	Wackenhuth & Levine (1977)
	Marine fouling	As effective as Cl_2, continuous worked better than intermittent application	Seawater, flow-thru cooling system	Burton & Margrey (1979)
Chloramine (monochloramine)	Asiatic clam (*C. flumines*)	Not tested against Cl_2, effective in the control of clams. Cu was better.	Freshwater,flow-thru, temp. dependent	Belanger et al (1991)
Chlorine Dioxide	Bacteria	As effective as Cl_2 no data on use in seawater	Review article	Waite & Fagan (1980)
	Freshwater fouling	More effective than Cl_2, similar results for continuous and intermittent applications	Comparison based on biofouling weight	Mayack et al (1984)
	Zebra mussel (*D. polymorpha*)	As effective as Cl_2, tested continuous application in freshwater	Tested at 15° C at two power plants	Khalanski (1993)
	Zebra mussel (*D. polymorpha*)	As effective as Cl_2, tested continuous application in freshwater	Several days in flow-through system	Matisoff et al (1991)
Hydrogen Peroxide	Mussel (*M.galloprovincialis*)	Not as effective as Cl_2, for 90% settlement inhibition, 1mg/L needed	90d test with reduced seawater flow rate	Nishimura et al (1988)
	Marine fouling	Not as effective as Cl_2, higher concentrations required	Review article	Waite & Fagan (1980)
	Mussel (marine)	Not as effective as Cl_2, higher concentrations required	60m pipe in seawater for 90d	Ikuta et al (1988)
	Zebra mussel (*D. polymorpha*)	Not as effective as Cl_2, much higher concentrations required	Freshwater, static, 12 and 22° C tested	Martin et al (1993a)
Hydrogen Peroxide + ferrous sulphate	Zebra mussel (*D. polymorpha*)	Not as effective as Cl_2, longer exposure time required	Freshwater, flow - through	Klerks et al (1993)
	Mussel (*M.galloprovincialis*)	As effective as Cl_2, inhibits larval settlement, not as toxic to environment	90d test with reduced seawater flow rate	Nishimura et al (1988)
	Mussel (marine)	As effective as Cl_2, inhibits larval settlement, not as toxic to environment	60m pipe in seawater for 90d	Ikuta et al (1988)
Iodine	Marine fouling	No data available, availability and cost are a problem	Review article	Waite & Fagan (1980)
Ozone	Marine fouling	Conflicting results, sometimes as effective as Cl_2, sometimes not	Review article	Waite & Fagan (1980)
	Mussel larvae (*Mytilus edulis*)	As effective at elevated temp., very temp. depend., older larvae more tolerant	Larvae were exposed to ozone treated seawater.	Toner & Brooks (1977)
Potassium Ferrate	Marine fouling	Little data available, possible availability problems, cost unknown	Review article	Waite & Fagan (1980)
Potassium Permanganate	Marine fouling	No seawater data, not as effective as Cl_2 in freshwater	Review article	Waite & Fagan (1980)
	Zebra mussel (*D. polymorpha*)	Not as effective as Cl_2, much higher concentrations required	Several days in flow-through system	Matisoff et al (1991)
	Zebra mussel (*D. polymorpha*)	Not as effective as Cl_2, more time and higher concentrations required	Freshwater, flow-through, intermittent and continuous	Klerks & Fraleigh (1991)

POTASSIUM PERMANGANATE (Table 3)

Potassium permanganate ($KMnO_4$) is a strong oxidant that has sparked considerable interest from the water treatment industry. It is an effective biocide for the control of a variety of algae and other slime-forming microorganisms. It reacts with all oxidizable material resulting in the precipitation of manganese dioxide. This brown precipitate, along with permanganate is quite toxic to aquatic organisms. It is much more effective in a low pH or acidic medium, losing its effectiveness at more natural pH levels. It does not oxidize chlorine or bromine, thereby eliminating the problem of halogenated organic compound formation.

Over the years, a number of freshwater studies have been completed on the zebra mussel (*Dreissena polymorpha*), at different stages of life. One study on mussel veligers, in a flow-through system, at reduced temperatures, found a continuous treatment at 1.0 mg/L produced a 60% mortality rate. A second treatment at 2.5 mg/L of potassium permanganate, produced a mortality rate of 64% (Van Benschoten et al., 1993). Klerks et al. (1993), investigated the use of several different oxidants to control zebra mussel veligers. They concluded that chlorine was better than permanganate and had a stronger effect on the survival of mussel veligers. This applied to both static and flow-through exposures. Klerks & Fraleigh (1991) used a similar experiment to determine the effects of several oxidants on adult (14-16mm) zebra mussels. They exposed the mussels to various concentrations of potassium permanganate over a 56-day period, using a continuous exposure, flow-through system. Permanganate dosing at 2.5 mg/L and 1.0 mg/L killed 50% of the mussels in 10.7 and 49.8 days respectively, while a permanganate concentration of 0.5 mg/L yielded no mussel mortality. They observed a significant decrease in filtering rate when the mussels were exposed to the oxidants, which seemed related to the concentration of the oxidant. They also noted a temperature effect, which they assumed was associated with mussel metabolism, and that continuous treatment was necessary for effective control. Although concentrations required for control, seemed relatively high, the authors concluded that potassium permanganate was a potential alternative for chlorine treatment. Another survey that used adult zebra mussels, exposed for several days in a flow-through system, concluded that >2ppm $KMnO_4$ was required to cause mortality (Matisoff et al., 1991).

Unfortunately, there seemed to be no information available for potassium permanganate treatment in seawater applications. There are probably a number of reasons for this: (1) effectiveness, being the most important (since permanganate reacts with all oxidizable material, seawater would certainly prove more demanding than freshwater); (2) toxicity to non-target organisms; (3) cost; and (4) solubility problems. Until more marine studies are carried out, it could not be recommended as an alternative to chlorine treatment.

CHEMICAL ANTIFOULANTS: NONOXIDIZING AGENTS

The next group of chemicals are termed nonoxidizing (Tables 4a-e), since generally their mode of action does not involve the oxidation of, the soft tissues of the target organism. This does not imply that the oxidation/reduction process may not occur, just that these chemicals are not classically considered oxidants. The exact mode of action for many of these chemicals is still not clear and will require further investigation before this is thoroughly understood. The categories used to classify the chemicals are arbitrary, and are simply for ease of presentation. Many may be considered for direct application, while others may only see use as paint additives. In many cases the method of chemical application was not clear, thereby making chemical categorization difficult.

Generally speaking, these chemicals (mainly organics) have found limited application as biofouling control agents. They have been used when certain conditions (chemical and/or biological) made the use of oxidants ineffective. Neutralization is commonly required because these chemicals are toxic to many non-target organisms. In many cases, the level of toxicity and the persistence of these chemicals in the environment, is not well understood. Little benefit is gained if the alternative is more harmful to the environment than the established treatment. The last problem associated with this class of biofouling control agents is cost. Many if not all, are more expensive than chlorine treatment, while few if any have proven more effective. The one advantage many of these compounds have over oxidants is the response of the mussel to exposure. The mussel does not normally recognize these compounds as noxious, therefore the mussel does not close its shell, resulting in quicker action of the antifouling agent.

The number of commercial products available is enormous, although most manufacturers are reluctant to release data on specific product applications, prohibiting the possibility of proper assessment. Website addresses (reference section) are provided for some of the companies and products available.

AMMONIA AND AMINES (Table 4a)

Ammonia (NH_3) and associated nitrogen compounds seem to comprise the largest group of nonoxidizing chemicals reported in the literature. A wide array of compounds (with varying degrees of effectiveness) fall into this category. The problem in assessing these compounds is that very little information is available pertaining to a marine biofouling application.

A few applications of ammonia, for the control of biofouling, have been documented. One freshwater study (Belanger et al., 1991), compared its effectiveness with oxidants, for controlling the Asiatic clam (*Corbicula fluminea*). Investigations

considered the effects of various concentrations and temperatures on clams from several different locations and stages of development. Results were reported as LT_{100} values, which is the time required for 100% mortality at the stated concentration. The following results were reported for the juvenile clams: 7.67 mg/L NH_3 gave an LT_{100} of 4.0 and 12.0 days for the two different locations. Adults exposed to the same concentration showed 100% mortality within 5.0 and 12.0 days. A second concentration of 15.6 mg/L was used and produced the following results: LT_{100} for juveniles was 4.0 and 8.0 days, and was 5.0 days for both sets of adults. The same study determined the effects of ammonia exposure on the pediveliger stage of the clam. This was done by exposing the veligers to ammonia for 48 hours and 20°C to determine the concentration that would cause 50% mortality (LC_{50}) of the veligers. The LC_{50} in this case was 1.72 mg/L for total ammonia. The authors concluded that effectiveness was temperature dependent, but was also influenced by the environmental history and genetics of the clam. Another study (Claudi & Mackie, 1994a) examined the effectiveness of ammonia nitrate in controlling zebra mussel (*Dreissena polymorpha*) infestation. The results showed that for adult zebra mussels, concentrations of 400 to 500 mg/L for 5 to 6 days (at 16-19°C) were required for 100% mortality. For 100% mortality of the mussel veligers, an excess of 3 mg/L was necessary. No information on ammonia treatment in marine applications was available.

Polyquaternary ammonium compounds are being considered for direct application and as additives to paint for controlling biofouling. Poly[oxyethylene (dimethylimino) ethylene (dimethyliminio) ethylene dichloride], is marketed under the trademark name of BULAB 6002 (Buckman Chemical Co.). The first freshwater study (Martin et al., 1993b), endeavored to determine the toxicity of BULAB 6002 using adult zebra mussels in a static system at room temperature (20-22°C). Zebra mussels were exposed to the following concentrations: 1.00, 2.00, 4.00 and 8.00 mg/L, yielding 50% mortality after 168, 148, 108 and 96 hours, respectively. A second study (Martin et al., 1993a), was performed to determine the effect of temperature on the effectiveness of BULAB 6002. Similar test conditions were used, except this time the experimental temperature was 12°C. Exposures were done at 1.0, 2.0 and 4.0 mg/L producing 50% mortality after 432, 290 and 264 hours, respectively. This illustrates the dependence on temperature when considering the effectiveness of BULAB 6002. Another group (McMahon et al., 1993) also looked at the toxicity of BULAB 6002 using adult (12-23mm) zebra mussels and adult (13-23mm) Asiatic clams in a static setting at 20 and 25°C, respectively. Zebra mussels were exposed to concentrations of 0.5, 1.0, 2.0, 4.0 and 8.0 mg/L producing 50% mortality in 700, 499, 216, 174 and 124 hours, respectively. The Asiatic clams were exposed to concentrations of 0.25, 0.50, 1.00, 2.00, 4.00 and 8.00 mg/L producing 50% mortality in 556, 256, 208, 54, 50 and 45 hours, respectively. This indicates that the toxicity of BULAB 6002 is also species dependent.

The next compound, an amine, is sold under the commercial name MEXEL 432 (www.mexel.fr/mexel432.htm). The composition is described as a substance comprised of open hydrocarbon chains with amines attached, containing about 80% water. The manufacturer claims that it is film-forming with dispersant activity. MEXEL 432 does not react with the raw water, but rather migrates to the surface of piping and forms a film and

in this way differs from many treatments. The only experimental data available, investigated the control of zebra mussel infestation at a power plant in France (Khalanski, 1993). A continuous injection at 7 ppm for 19 hours caused 95% mortality of the mussels after 4 days.

Another commercial molluscicide, marketed as H-130 (didecyl dimethyl ammonium chloride), is also a quaternary ammonium compound that is positively charged. H-130 has seen limited use in freshwater, as a controlling agent against zebra mussel infestation. The study (Bargar & Fisher, 1997) examined (1) the toxicity of H-130 to non-target organisms, and (2)whether bentonite clay could adequately detoxify H-130. The study indicated that H-130 was toxic to three non-target organisms at relatively low concentrations and that bentonite clay was unable to completely eliminate the toxic effects of H-130. Another report (Barton, 1993) investigated the use of didecyl dimethyl ammonium chloride as well as a blend of alkyl dimethyl benzyl ammonium chloride and dodecyl guanidine hydrochloride, to control freshwater zebra mussel infestation. Both products were used in an end of season application and were neutralized with bentonite clay before discharge. Depending on the product and water temperature, applications consisted of 6-12 hours at concentrations of 2.5-15.0 mg/L, which resulted in a mortality rate over 90% for the mussels in a flow-through system.

Two other studies investigated the antifouling properties of benzalkonium chloride (also known as alkyldimethylbenzylammonium chlorides) incorporated into a coating for antibacterial purposes. One study, concluded the potential for benzalkonium chloride as an antifoulant was good, based on its results from other applications (Parr et al., 1996). The authors also noted that benzalkonium chloride appeared to be an environmentally acceptable product. His et al. (1996), concluded that benzalkonium chloride, incorporated into a polymer film, was not toxic to the non-target organism tested, and extended the antifouling properties of the film.

The last polyquaternary ammonium compound to be considered is 1,1'-(methyliminio)*bis*(3-chloro-2-propanol), polymer cross-linked with *N,N,N',N'* tetramethyl-1,2-ethanediamine. The freshwater, static study (McMahon et al., 1993) was carried out at 20°C on both juvenile (4-11mm) and adult (13-27mm) zebra mussels. The juvenile mussels were exposed to concentrations of 3.0, 5.0, 7.0 and 9.0 mg/L, which produced 50% juvenile mussel mortality in 875, 374, 290 and 197 hours, respectively. The adults were exposed to identical concentrations which yielded 50% mortality after 946, 609, 516 and 532 hours, respectively. From the results, the effectiveness of the compound seems to be related to size or age of the mussel.

DBNPA (2,2-dibromo-3-nitrilopropionamide) is another nitrogen-containing compound that is sold as a biocide. It has found use in both recirculating and once-through cooling water systems. Application in a once-through system would typically be for several hours once a week. This biocide has been successful in controlling biofouling in some applications, although concerns about its environmental effects continue to grow (Klaine et al., 1996). Even at relatively low concentrations, DBNPA is quite toxic to

numerous non-target organisms and does not appear to readily degrade in freshwater. Again, there is a lack of information regarding marine applications.

A recent study (Finlay & Callow, 1996) investigated the toxicity of 18 alkyl amines on two species of marine algae and one marine invertebrate larvae. The amines tested were of four general structures: linear alkyl primary, linear alkyl secondary, tertiary amines, and dimethyl alkyl tertiary amines. All the tests were carried out in static, artificial seawater at 20-25°C, for 24-96 hours. The results were complex, making interpretation difficult. The control of the three test organisms was varied, which implies toxicity is not solely dependent on chemical structure. Overall, the authors concluded that the best candidates, of the 18 amines tested, for controlling the whole spectrum of fouling organisms, would be either dioctylamine or dimethyldodecylamine. This looks more promising since the experiments were undertaken in a saltwater environment. Solubility and cost could curtail any consideration of these compounds, while mode of application, environmental effects and toxicity to non-target organisms remain unanswered questions.

It is apparent that there are a tremendous number of amine compounds presently under consideration for use as biocides or antifoulants. A brief examination of the number of patent applications for marine antifouling agents globally, will confirm this. The website addresses for US patents are provided in the reference section. Both examples are patents for marine antifouling agents that are being considered as paint additives. The first is a group of thione maleimides (dimercapto-1,3-dithiolo-2-one) were tested against panels that were treated with tributyl tin oxide (TBTO) for 6-10 weeks in a marine environment. The test paint exhibited some antifouling properties, but was not as effective as the TBTO treated panels (Wu et al., 1995). The second example involved substituted thiadiazoles compounds, that were tested in the same manner as the thione maleimides, with panels immersed for 6-10 weeks. Some antifouling properties were displayed by the test compounds, but they were not comparable to the level of protection provided by the TBTO treated panels (Shanker et al., 1996).

CARBAMATE COMPOUNDS (Table 4a)

We were able to locate the names of a few of these compounds from an Internet search. The two compounds are potassium dimethyl dithiocarbamate and potassium n-methyldithiocarbamate. They were both available in a liquid form and were listed as marine antifoulants (www.cdpr.ca.gov/cgi-bin/label/labchemrep.pl). Until much more information is provided about effectiveness, cost, safety and environmental impact, it would be impossible to consider these compounds as possible alternatives to chlorine treatment.

CYANO COMPOUNDS (Table 4a)

The last group of compounds to be considered in this section are the cyano-containing (CN^-) compounds. Many cyano-containing compounds have been considered as antifoulants over the years, with limited success. One such compound is 2-(thiocyanomethylthio) benzothiazole (BULAB 6009, Buckman Chemical Co.), which was tested in a couple of published studies.

The first study (Martin et al., 1993b) examined the effects of 2-(thiocyanomethylthio) benzothiazole in static, freshwater, on juvenile (mean 3.75mm) zebra mussels (*Dreissena polymorpha*), at various concentrations. The room temperature experiments used concentrations of 0.50, 1.00, 2.00 and 4.00 mg/L, which produced 100% mortality of the mussels in 192, 144, 144 and 110 hours respectively. The study indicated that the test compound was more effective than sodium hypochlorite, which was also tested under the same conditions. In a second static, freshwater study, McMahon et al. (1993), studied the effects of 2-(thiocyanomethylthio) benzothiazole, at various concentrations, on the adult zebra mussel and Asiatic clam (*Corbicula fluminea*). The zebra mussel was exposed to concentrations of 0.50, 1.00, 2.00 and 4.00 mg/L that yielded 100% mortality in 758, 485, 313 and 260 hours, respectively. While the clam was exposed to concentrations of 0.125, 0.250, 0.500, 1.00, 2.00 and 4.00 mg/L producing 100% mortality of the clams in 1411, 735, 566, 160, 127 and 120 hours, respectively. This particular compound proved quite effective in its freshwater applications.

Slimicide C-30, another cyano-containing compound, is described as a synergistic mixture of halogenated organic sulphur biocide and methylene bistiocyanate, consisting of *bis*(trichloromethyl) sulfone (20%), methylene bisthiocyanate (5%), and unspecified ingredients (75%). It was tested, in static seawater, on mussel (*Mytilus galloprovincialis*) embryos at various concentrations (0.005-0.200 mg/L), to study the effects of 96-hour exposure. Lucu et al. (1980), concluded that 0.07 mg/L was the concentration at which Slimicide C-30 inhibited development of 50% of the mussel embryos. The work was undertaken due to a toxicity concern with Slimicide C-30 use. The authors indicated that the compound seems quite resistant to decomposition at elevated (38°C) temperatures, but no field tests were conducted. This compound is toxic to marine mussel embryos at relatively low concentrations, leading to a concern about toxicity to non-target organisms and environmental impacts.

The only other cyano-containing compound listed in the literature is disodium cyanodithioimido carbonate. It appears on a list of chemicals from an Internet search for marine antifoulants. It is available in a liquid form. The website address (www.cdpr.ca.gov/cgi-bin/label/labchemrep.pl) is also provided in the reference section.

Table 4a: Nonoxidizing Agents- Organics

Chemical Type	Compounds Tested	Species Tested	Observations	Notes	Source
Ammonia and amines	ammonia	Asiatic clam (*C. fluminea*)	Nearly as effective as Cl_2, works best in combination with NH_2Cl	Freshwater, temperature dependent	Belanger et al (1991)
	BULAB 6002[1]	Zebra mussel (*D. polymorpha*)	As effective as Cl_2 in continuous application but not intermittently	Freshwater, very temperature dependent	Martin et al (1993a)
	MEXEL 432[2]	Zebra mussel (*D. polymorpha*)	Almost as effective as Cl_2, environmental effects unknown	Freshwater, continuous flow	Khalanski (1993)
	alkyl amines (18)	Crustacean (larval) (*Artemia salina*)	Intercomparison of 18 amines, dioctyl, dimethyldodecyl best	Artificial seawater, 24h experiment at 25° C	Finlay & Callow (1996)
	substituted thiadiazoles	Marine fouling	Encouraging results, very little data	Seawater tests 6-8 wks, in paint on test panels	Shanker et al (1996)
	thione maleimides	Marine fouling	Preliminary results not that encouraging, very little data	Seawater tests 6-10 wks, in paint on test panels	Wu et al (1995)
	benzalkonium chloride[3]	Bacteria	Works well against many types of bacteria.	Many potential problems with marine application.	Parr et al (1996)
	benzalkonium chloride	Marine fouling	Results somewhat encouraging, low toxicity to marine organisms	Antimicrobial agent added to coating, very preliminary	His et al (1996)
Carbamate compounds	potassium dimethyl dithio carbamate		No data	Listed as anti-foulant(marine)	Internet (see ref.)
	potassium n-methyldithio carbamate		No data	Listed as anti-foulant(marine)	Internet (see ref.)
Cyano compounds	SlimicideC-30[4]	Marine mussel (*M. galloprovincialis*)	Quite toxic to larvae at low concentrations	Static seawater at 13° C, rate of decomposition unknown	Lucu et al (1980)
	BULAB 6009[5]	Zebra mussel (*D. polymorpha*)	Very effective, also very toxic to non-target organisms	Static freshwater at 22° C, young adults (2-8 mm)	Martin et al (1993b)
	disodium cyanodithioimido carbonate		No data	Listed as anti-foulant(marine)	Internet (see ref.)

BULAB 6002[1]: poly[oxyethylene (dimethylimino) ethylene (dimethylimino) ethylene dichloride]
MEXEL 432[2]: open hydrocarbon chains containing amines + 80% water
benzalkonium chloride[3]: mixture of alkyldimethylbenzylammonium chlorides
SlimicideC-30[4]: *bis* (trichloromethyl) sulfone (20%), methylene bisthiocyanate (5%)
BULAB 6009[5]: (2-(thiocyanomethylthio) benzothiazole)

METAL SALTS (Table 4b)

This section considers the use of metal salts as antifoulants, without including the organometallics and metallic paint additives, which will be discussed later. Although many salts have been tested, discussion will include only the metal salts which exhibit some effect on the overall health of the test organism. With growing concern about metal pollution, many studies (i.e. Mussel Watch) have investigated the effects on various organisms. Much of this work was warranted, although it is not possible to cover it all, so discussion will be limited to the relevant material.

ARSENIC (As)

Natural concentrations of arsenic in marine, open waters are generally quite low, typically between <1 and 3 ppb, although near-shore levels can be several times higher. One study (Unlu & Fowler, 1979), examined the effect of temperature and salinity on the uptake of arsenic (Na_3AsO_4) by the adult (2-14 g), marine mussel (*Mytilus galloprovincialis*) exposed to natural levels (~2 ppb). The 20 day, static experiment showed that uptake was not proportional to arsenic concentration in the seawater. Increasing temperature enhanced both arsenic uptake and elimination, while decreased salinity increased accumulation and retention. The authors concluded that the relationship between arsenic in water and uptake by the mussel was complex and uptake was not proportional to concentration. Another study (Martin et al., 1981), investigated the effect of arsenic (As_2O_3) exposure to developing embryos of the mussel (*Mytilus edulis*) and oyster (*Crassostrea gigas*) through short-term (48 hours) static bioassays (17-20°C). Results were based on the normal or abnormal development of the embryos. The researchers determined the concentration of arsenic that would cause 50% of the embryos to develop abnormally. These concentrations were 326 ppb for the oyster and >3000 ppb for the mussel. At this stage mussel embryos were more resistant to arsenic exposure than oyster embryos.

Based on the presented results, arsenic would not be the metal of choice for controlling marine mussel infestation. The levels necessary for control of mussel infestation, would prove lethal to many other non-target organisms.

CADMIUM (Cd)

Cadmium is another metal that is acutely toxic to many organisms. The natural levels in open, seawater are quite low, ranging from 0.01 ppb to 0.1 ppb, although they can be higher in near-shore and estuarine sites. The effects of cadmium on the health and

viability of many marine organisms has been thoroughly examined. Phillips (1976a) investigated how the uptake of cadmium ($CdCl_2$), by marine mussels (*Mytilus edulis*), was affected by environmental variables and the presence of other metals. He determined that seasonal variation had an effect on uptake and that low salinity increased the uptake of cadmium. He showed that cadmium bioaccumulation decreased at low water temperature and salinity, and also that the presence of other metals did not affect cadmium accumulation in adult (40-47mm) *Mytilus edulis*. In a follow-up report, Phillips (1976b), concluded that cadmium uptake was fairly independent of a wide variety of naturally occurring environmental conditions.

Maung Myint & Tyler, (1982), studied the affect of temperature and metal exposure on the reproductive status of adult (43-46mm) mussels (*Mytilus edulis*). They found that decreased temperature slows gonadal development, but does not stop the process completely. Of the metals tested, cadmium (as $CdSO_4$, with 50 ppb Cd) was the least toxic, with a linear uptake mechanism which resulted in relatively high accumulation. They noted, that even with high accumulation of cadmium, the mussels appeared reasonably healthy. This fact was also mentioned in a study (Roberts et al., 1986) of growth effects in adult (35-40mm), marine mussels (*Mytilus edulis*) from an area of known anthropogenic input. They reported that accumulation decreased proportionally with increased distance from the cadmium source (industrial effluent), and that increased tissue Cd levels did not affect growth rates. Growth rate was the parameter used to calculate the affects of cadmium ($CdCl_2$), in a short-term (10-22 days), flow-through exposure of adult (19-29mm), marine mussels (*Mytilus edulis*) at relatively low (8.7°C) temperature. Interestingly, the results indicated that shell growth was stimulated at cadmium concentrations of 2 ppb and lower, while shell growth was reduced at cadmium levels of 5-10 ppb. Stromgren (1982), concluded that exposure to a cadmium level of 100 ppb would result in a 50% reduction in shell growth.

Martin et al. (1981), examined the toxicity of ten metals to the embryos of several different marine invertebrates. The results were based on the normal development of the embryo during exposure to cadmium ($CdCl_2$) for 48 hours in static seawater. The concentration of cadmium that resulted in abnormal development in 50% of the embryos was 1200 ppb for the marine mussel (*Mytilus edulis*) and 611 ppb for the marine oyster (*Crassostrea gigas*).

By comparison, adult marine clams (*Mya arenaria*) were exposed to cadmium ($CdCl_2$) at a concentration of 1 ppb in a flow-through system for 112 days. Depending on temperature, the metal mixture proved toxic, but examination of the clam soft-tissue revealed virtually no accumulation of cadmium. Eisler (1977), concluded that the mortalities were caused by other metals in the mixture.

Considering the above information, it is clear that the use of cadmium in controlling mussel infestation would not be advisable. The concentrations required to control mussels would likely have a much greater detrimental affect on non-target organisms than on the marine mussels.

CHROMIUM (Cr)

Studies concerning chromium exposure and mussel toxicity, were generally initiated as a result of the disposal of anthropogenic waste. In one such study, Martin et al. (1981), investigated the toxic effect of ten metals on several marine invertebrate embryos. The results of these short-term (48-hours) toxicity tests indicated that a concentration of 4469 ppb chromium (K_2CrO_7) was necessary to produce 50% abnormal larval development in mussel (*Mytilus edulis*) embryos and 4538 ppb Cr was required for oyster (*Crassostrea gigas*) embryos. As mussel embryos are tolerant to rather high concentrations (albeit short-term) of chromium, this element would be a poor choice for the control of mussel infestation. Since earlier life stages are generally more susceptible to chemical treatment, higher treatment concentrations would likely be required for the control of adults.

COPPER (Cu)

In seawater, normal copper levels are quite low, ranging from 3 to 10 ppb. Copper toxicity in the marine environment has been well-documented over the years. Many marine organisms find copper toxic at rather low levels. For this reason, copper has found many applications in the antifouling industry (i.e. sheathing, paint additive).

Copper is an essential element for mussel metabolism (White & Rainbow, 1985), however, it can be toxic at elevated levels. Although essential, copper regulation in the mussel (*Mytilus edulis*) is incomplete. Beyond a certain exposure level, the mussel does not have the capacity to regulate copper accumulation, and this is when the metal can have sub-lethal or lethal effects (Harrison et al, 1983).

Research in this area dates back many decades. In a study of copper toxicity to adult mussels in static seawater at 10°C, Scott & Major (1972), observed a depressed rate of mussel respiration as the level of copper exposure increased. Tests indicated a threshold toxicity of 100-200 ppb Cu for mussels, although in 7 days, at a concentration of 300 ppb Cu, only 55% of the mussels died. Due to discrepancies with other published data, the authors concluded that the mussel had some method of detoxification, possibly aided by mucous secretion. In an Australian study, Phillips (1976a), investigated the effects of environmental variables on the accumulation of metals (copper as $CuCl_2$) in adult (40-47mm) mussels. Study results indicated that copper uptake was erratic, influenced to some degree by season, salinity, temperature and the presence of other metals. The authors concluded that uptake kinetics for copper differed from most metals accumulated by the mussel. A further study of mussels of the same size range, was conducted in the field (Phillips, 1976b). Mussels sampled at various distances from known discharge sites, contained similar copper levels in soft tissues, indicating different uptake kinetics and/or some form of regulation.

Davenport (1977), studied the effects of differing application methods of copper ($CuSO_4$), with fluctuating salinity levels on the adult (40-50mm) mussel. The tests were carried out at 15°C, in a flow-through seawater system, with continuous application of copper. Concentrations of 250 and 500 ppb of Cu produced MLT's (median lethal time) of 4-5 and 2 days, respectively, for the mussels exposed. The intermittent application (500 ppb) consisted of, 6 hours of copper application, followed by 6 hours of no copper. After 5 days of this intermittent treatment, the mussels appeared unaffected, due to the valve closure response. The same response was observed during fluctuating salinity experiments, and the author hypothesized that intermittent copper treatment coupled with fluctuating salinity, might trick the mussel into opening during copper treatment. The valve closure response warranted further study. This investigation (Davenport & Manley, 1978) involved copper ($CuSO_4$) exposure, in a flow-through seawater system, at 15°C, using adult (30-60mm) mussels. Some mussels were first used to determine an acute toxicity threshold, which was 90-100 ppb. Valve closure response to elevated copper levels was then explored. The process involved several stages: the initial closure occurred at 21 ppb Cu, and complete closure occurred around 200 ppb Cu. If the mussel was first acclimatized to slightly elevated copper levels, the initial closure response occurred at relatively higher copper levels. The same phenomenon had been observed earlier with temperature and mussel acclimatization.

In a study of the vulnerability of mussels to copper exposure during the embryonic stage of development, Martin et al. (1981), exposed mussel embryos to copper ($CuSO_4$) in a static, seawater experiment for 48-hours. They reported that 5.8 ppb Cu caused 50% of the embryos to develop abnormally, illustrating the sensitivity of mussel embryos to low levels of copper. This concentration is considered a natural level in some marine locations.

The effects of heavy metal exposure to growth were investigated (Stromgren, 1982). Stromgren used a flow-through, seawater system operated over a range of temperatures (7.7-15.2°C), and exposed adult mussels (19-29mm) for a period of 10-22 days to various copper ($CuSO_4$) concentrations. The author reported that permanent valve closure occurred at 5 ppb Cu, growth ceased at 8-10 ppb, but no mortality occurred after 3 weeks. Lack of growth is likely related to valve closure, since feeding cannot take place if water filtration halts. Results also showed that at copper levels of 40-80 ppb, mussel mortality was 50% over a 14 day period. Another study (Maung Myint & Tyler, 1982) explored the effects of temperature and metal exposure on adult mussels (43-46mm) in a static, seawater experiment. Of the metals tested, copper ($CuSO_4$) had the lowest uptake, but was the most toxic. Temperature experiments, using 50 ppb Cu, administered for 14 days at 18°C, produced 55% mortality in the exposed mussels. Lowering the temperature to 0°C, while maintaining all other experimental parameters, resulted in no mortality over a 70-day period. The authors observed that lower temperatures slowed the reproductive process.

Manley (1983) investigated the sublethal effects of copper exposure on behavior and physiological processes in the adult (65-70mm) mussel. A flow-through, seawater system (at 14°C) was used in the copper ($Cu(NO_3)_2$) exposure tests. The isolation (valve closure) response was observed, and had a negative effect on several physiological processes. Results were a little ambiguous, but there was a definite negative effect on mussel respiration, filtration and ventilation, in response to copper exposure. Another study by Harrison et al. (1983), with regard to the sublethal effects of copper reported the apparent detoxification process undertaken by the mussel during copper exposure. Harrison et al. described the existence of metal-binding proteins that aid in the detoxification process, to a certain extent. If exposure was long enough, however the process eventually failed, and mussel mortality followed. Research also addressed the sublethal effects of copper on mussel shell growth (Manley et al., 1984). Using a flow-through, seawater (at 14°C) system, adult (10-15mm) mussels were exposed to various concentrations of copper. Exposure to 10 ppb Cu, showed inhibitory affects on shell growth within 3 days. As time passed, this inhibition of shell growth continued until growth essentially ceased. If returned to untreated seawater, normal mussel shell growth rate was restored.

Long-term (21- months) experiments, performed in a flow-through system (temp. range 3-24°C), exposed adult (4-5mm) mussels to various concentrations of copper or silver. Calabrese et al., (1984), reported that the accumulation (nonlinear) of copper increased (in the soft-tissue) as copper exposure concentration increased. The authors stated that copper (10 ppb) inhibited growth, interrupted normal feeding and digestion processes, and ultimately led to mortality. The experimental results also showed that mussels accumulate more copper in the presence of silver. There seemed to be a synergistic process occurring for copper accumulation in the presence of silver. Research continued to examine growth inhibition and the apparent recovery mentioned earlier. Redpath (1985) used a flow-through, sea water (at 14°C) system, to examine growth inhibition by copper ($CuCl_2$) exposure over a 10-day period. He determined that significant growth inhibition occurred at >2 ppb copper and 50% growth inhibition occurred at 6 ppb Cu. When returned to untreated seawater, the mussel growth rate returned to almost normal levels.

Beaumont et al. (1987), studied the tolerance of mussel larvae (veligers) to elevated copper levels. The 15-day experiment employed a static, seawater (at 15°C) system for copper ($CuCl_2$) exposure at various concentrations. The authors reported that the copper concentration that caused a 50% mortality of the mussel larvae, in 15 days, was 400 ppb. Comparison with other published data led the authors to conclude that the mussel veligers were 7 to 10 times more tolerant than adults to copper exposure.

It is obvious, from all the presented information, that copper is very effective at controlling mussel infestation. The effects of copper exposure on the mussel are varied and often complex, differing from many other metal-mussel interactions. Copper would be a reasonable alternative to chlorine treatment, except that it suffers from many of the same drawbacks as chlorine. It is very toxic to many non-target organisms at

concentrations lower than that necessary to cause mussel mortality. At equivalent concentrations (to chlorine), copper treatment would be considerably more expensive. If all the prospective biofouling control agents and methods were as well documented as copper, however, the selection process would be much easier.

LEAD (Pb)

The toxicity (lethal and sublethal) of this metal has been well documented since many organisms have a low lead tolerance. The wide-ranging toxicity coupled with the tendency to bioaccumulate in organisms, has resulted in many concerns over lead contamination. These concerns apply to humans since the organisms that accumulate lead are often consumed as food products, passing on much of the accumulated lead. For this reason many commercial and industrial applications have ceased using lead (i.e. gasoline additive).

Martin et al., (1981), examined the toxicity of lead to mussel (*Mytilus edulis*) and oyster (*Crassostrea gigas*) embryos in short-term (48-hour), static, seawater (at 17-20°C) experiments. It was determined that the concentration of lead ($Pb(NO_3)_2$) required to cause abnormal development in 50% of the embryos was 476 ppb for mussel and 758 ppb for oyster. A previously mentioned study (Phillips, 1976a), investigated the environmental effects on the uptake of metals by the adult (40-47mm) mussel (*Mytilus edulis*). Lead accumulation was unaffected by seasonal changes in mussels collected from the field. In laboratory experiments (static seawater) the effects of salinity, temperature and metal-interaction on the uptake of lead ($Pb(NO_3)_2$) were investigated. Net lead uptake decreased at lower salinities, but remained unaffected by lower temperatures or the presence of other metals. An ensuing field study investigated the ability of the mussel (*Mytilus edulis*) to reflect environmental levels of metal contamination. Since lead uptake was not strongly influenced by environmental effects (i.e. salinity, water temperature), the mussel accumulated lead proportional to local seawater Pb concentrations (Phillips, 1976b).

Majori & Petronio (1974), examined the uptake and excretion rates of lead from a polluted environment by adult mussels (*Mytilus galloprovincialis*). The authors derived an equation to illustrate the dependence of lead uptake on concentration, but were unable to derive an equation for the excretion rate. They also estimated that the lethal concentration of lead for mussels was >200 ppb. The sublethal effect on growth, of short-term (10-22 days) exposure to lead ($Pb(C_2H_3O_2)_2$), was studied in the adult (19-29mm) mussel (*Mytilus edulis*). This flow-through, seawater (at 10°C) experiment used various concentrations (5-200 ppb) of lead, but even after 8 days at the highest concentration (200 ppb), treated mussels showed no difference in behavior or growth rate compared with controls (Stromgren, 1982). By comparison, metal accumulation by the marine clam (*Mya arenaria*), exposed to a metal mixture in flowing seawater, resulted in a final

concentration (soft-tissue) of 70 ppb lead (Eisler, 1977). After 7 days of exposure to the treated seawater, a 24-fold increase in soft tissue lead concentration was observed. The author attributed mortalities to copper in the metal mixture. The rapid uptake of lead appears to be common in a number of marine invertebrates.

MANGANESE (Mn)

In recent years, the use of this metal in industry has increased, along with concerns over environmental issues. Few studies regarding the toxicity of manganese to marine organisms have been reported. Eisler (1977), exposed marine clams (*Mya arenaria*) to a mixture of metals, and reported that the clam does accumulate manganese, but no mortality was attributed to the accumulation of this metal.

MERCURY (Hg)

Mussel (*Mytilus edulis*) and oyster (*Crassostrea gigas*) embryos are quite sensitive to mercury. In a short-term (48 hours) study of toxicity, embryos were exposed (at 17-20°C) to ten different metal salts individually. Martin et al. (1981), reported the mercury ($HgCl_2$) concentration that caused abnormal development in 50% of the embryos was 5.8 ppb for the mussel and 6.7 ppb for the oyster. Only copper proved as toxic as mercury to the mussel embryos.

Calabrese et al. (1977), examined the toxicity of mercury ($HgCl_2$) and other metals to the larvae of the oyster (*Crassostrea virginica*) and the clam (*Mercenaria mercenaria*). Tests were performed in static seawater (25°C) for a period of 8 to 12 days. The mercury concentration necessary to cause 95% mortality of the larvae was 20.7 ppb for the oyster and 25.4 ppb for the clam. It was the most toxic element tested (including copper) for both organisms.

Stromgren (1982) investigated the mortality and growth of adult (19-29mm) mussels (*Mytilus edulis*) exposed to a mixture of metals for 10-22 days, in a flow-through system utilizing raw seawater (8-9°C). Mercury ($HgCl_2$) was continuously administered at various (0.3-50 ppb) concentrations, producing a reduced growth rate in the mussel even at the lowest concentration (0.3 ppb). At concentrations of mercury above 1.6 ppb, growth almost ceased within 3 or 4 days. At 25 ppm Hg, acute lethal effects were observed within 24 hours. Results indicated that a concentration of 0.3-0.4 ppb Hg caused a 50% decrease in the mussel growth rate.

The acute toxic response to mercury is common in many organisms. The health and environmental concerns over mercury exposure are well known and documented. Like lead, there is an ever-growing concern regarding the potential contamination of environmentally sensitive habitats. Thus, the use of a mercury salt in controlling biofouling, could never be considered in a flow-through, water cooling system, without recovery of the mercury before discharge of the cooling water.

NICKEL (Ni)

Nickel has gained attention as a result of its environmental impacts, particularly with the introduction of this metal (into the environment) from anthropogenic sources. The toxicity (lethal and nonlethal) information available for this metal is limited. However, recent publications indicate that nickel is not acutely toxic.

In one study, mussel (*Mytilus edulis*) and oyster (*Crassostrea gigas*) embryos were exposed to a number of individual metal salts (Martin et al., 1981). These short-term (48-hour), static experiments, used seawater (at 17-20°C) dosed with a range of nickel ($NiSO_4$) concentrations to examine toxicity to the embryos. The nickel concentration which produced abnormal development in 50% of the embryos was 891 ppb for mussels and 349 ppb for oysters.

Another study (Calabrese et al., 1977) examined the effects of nickel exposure on the larval stage of the American oyster (*Crassostrea virginica*) and clam (*Mercenaria mercenaria*). Experiments were accomplished in static, seawater, at 25°C, over a period of 8 to 12 days. The concentration of nickel ($NiCl_2$) that caused a 95% mortality rate in the larvae (in 8-12 days) was estimated at 2500 ppb for oyster and 10300 ppb for clam. Obviously, invertebrate larvae are much more resilient to nickel exposure than the associated embryonic stage.

A study (Friedrich & Filice, 1976) designed to examine the uptake and accumulation of nickel by the adult mussel, *Mytilus edulis*, (27-33mm) used static, artificial seawater (at 12-13°C). Nickel ($NiCl_2$) exposure was examined at various concentrations (20-80 ppm) for the 96-hour tests. At higher Ni concentrations (>40 ppm), byssal thread production decreased. This distinct decrease was attributed to mussel valve closure during most of the experimental period. Four-week exposure experiments, indicated an accumulation rate which was dependent (somewhat linear relationship) on concentration. Stromgren (1982), investigated the toxicity and growth effects of nickel ($NiCl_2$) on the adult blue mussel (19-29mm), using flow-through seawater (at 6.8°C) with nickel dosing at various concentrations (10-200 ppb) over 10 to 22 days. He reported that even at 200 ppb Ni for 8 days, no significant change in behavior or growth rate of the mussels was observed.

Nickel is much less toxic than copper or mercury when considering mussel mortality. Information on the environmental impact of nickel is still quite limited, as these studies usually require a large time frame.

POTASSIUM (K)

Potassium has freshwater applications in the control of zebra mussel infestations. Fisher et al. (1991), determined that a single dosing of potassium (138-226 mg/L) resulted in 50% mortality of the adult (15-20mm) zebra mussels in 24 hours at 20°C. Lewis et al. (1997), reported that 100 mg/L of potassium continuously administered to adult zebra mussels, resulted in mussel mortality of 91-100% within four days. Since potassium is naturally present in seawater at relatively high concentrations, the addition of potassium would have to be in enormous quantities to affect the target organisms.

SELENIUM (Se)

The only documented study found on the effects of selenium exposure to mussels, was carried out on the embryonic stage. Martin et al. (1981), used short-term (48 hours), static, seawater (17-20°C) tests to examine the toxicity of selenium (SeO_2) to mussel (*Mytilus edulis*) and oyster (*Crassostrea gigas*) embryos. A selenium concentration in excess of 10,000 ppb would cause 50% of both types of embryos to develop abnormally, in a 48-hour exposure. Based on previous information, higher concentrations would likely be necessary to control adult mussels.

SILVER (Ag)

Silver is a familiar metal due to its monetary value. Compounds containing silver are used as preservatives in a number of products and have medicinal uses dating back centuries (Gupta & Silver, 1998). Concern has increased over the years as silver contamination has become a larger environmental matter, prompting a number of studies.

In a study of silver toxicity to invertebrate embryos, Martin et al., (1981), examined the effects on mussel (*Mytilus edulis*) and oyster (*Crassostrea gigas*). Short-term experiments in static seawater were carried out at 17-20°C, using various silver ($AgNO_3$) concentrations. 14 ppb Ag (mussel), and 22 ppb Ag (oyster) caused half of the embryos to develop abnormally in a 48-hour exposure period. Invertebrate larvae seem to

share sensitivity to silver with invertebrate embryos. Experimental results from a study on the toxic affects of silver to oyster (*Crassostrea virginica*) and clam (*Mercenaria mercenaria*) larvae verify this sensitivity (Calabrese et al., 1977). Tests were performed in static seawater (25°C) for a period of 8-12 days, using various concentrations of silver ($AgNO_3$). To cause a 95% larval mortality rate, 35.7 ppb Ag for oyster and 46.2 ppb Ag for clam larvae were required.

In the mussel (*Mytilus edulis*), there is an apparent relationship between silver and copper. A long-term study on silver exposure to adult mussels in a flow-through seawater system, showed that copper accumulation increased with increasing silver concentration, even without the addition of copper to the seawater (Calabrese et al., 1984). Somehow the uptake mechanisms are inter-related or one metal aids in the absorption of the other. The reasons are still not understood, but this is a good example of how the presence of one metal affects the actions (i.e. accumulation) of another.

Silver seems to be toxic enough to the mussel, but how toxic is it to non-target organisms? This question would require consideration along with the potential environmental impacts associated with silver treatment. Even if adequate answers could be provided for the above concerns, silver treatment would prove a costly alternative to existing biofouling control techniques.

ZINC (Zn)

The last of the metal salts to be considered, shares some common characteristics with copper. First, a considerable volume of literature is available on zinc in the marine habitat. Secondly, like copper, Zn is an essential metal in marine mussels (White & Rainbow, 1985). However, zinc, unlike copper, is not acutely toxic to most marine organisms. Much of the work involving zinc accumulation in mussels was motivated by the prospect of using the mussel as an environmental monitor.

Embryonic sensitivities of mussels (*Mytilus edulis*) and oysters (*Crassostrea gigas*) to various concentrations of zinc ($ZnSO_4$) were determined in a short-term, static seawater experiment, performed at 17-20°C (Martin et al., 1981). The concentration which caused 50% of the embryos to develop abnormally, was 175 ppb Zn for the mussel, and 119 ppb Zn for the oyster. Calabrese et al. (1977) investigated the survival of marine clam (*Mercenaria mercenaria*) larvae exposed to elevated zinc levels. They reported that exposing clam larvae to 341 ppb Zn, for a period of 8 to 10 days, under static seawater condition at 25°C, resulted in a 95% mortality rate.

The effects of zinc exposure on the adult mussel (*Mytilus edulis*) have been more thoroughly researched in view of their monitoring potential. In an Australian study, Phillips 1976a, conducted laboratory and field experiments to examine the environmental

Table 4b: Nonoxidizing Agents- Metals

Metal	Salt Used	Species	Age	Temp. (°C)	Duration	Conc.	Addition Method	Results	Notes	Author
Arsenic	Na_2AsO_4	Mussel (*M. galloprovincialis*)	Adult (2-14 g)	12	10 days	~ 2 ppm	Natural levels	~ 5.8 CF[1]	Seawater, static	Unlu & Fowler (1979)
				21	10 days	~ 2 ppm	Natural levels	~ 11 CF	Seawater, static	
	As_2O_3	Mussel (*Mytilus edulis*)	Embryos	17	48 hours		Single dose	3000 ppb (EC_{50}[2])	Filtered seawater, static	Martin et al (1981)
		Oyster (*Crassostrea gigas*)						326 ppb (EC_{50})		
Cadmium	$CdCl_2$	Clam (*Mya arenaria*)	Adult (5g av meat wt)	16-22	14 days	0.2 ppb	Continuous	0.6 ppm (dry wt)	[Cd] unchanged from control, seawater, flow-through	Eisler (1977)
	$CdCl_2$	Mussel (*Mytilus edulis*)	Adult (44-47mm)	18	35 days	20 ppb	Dosing as required	6.8 ppm (wet weight)	Seawater, static, water changed every 2 days.	Phillips (1976a)
						40 ppb		6.7 ppm (ww)		
	unknown	Mussel (*Mytilus edulis*)	Adult (~40mm)	Aug/Sep		0.3 kg/d	Discharge	0.97 ppm (ww)	From polluted harbour	Phillips (1976b)
Chromium	K_2CrO_7	Mussel (*Mytilus edulis*)	Embryos	17	48 hours		Single dose	4469 ppb (EC_{50})	Filtered seawater, static	Martin et al (1981)
		Oyster (*Crassostrea gigas*)						4538 ppb (EC_{50})		
Copper	$CuSO_4$	Mussel (*Mytilus edulis*)	Embryos	17	48 hours		Single dose	5.8 ppb (EC_{50})	Filtered seawater, static	Martin et al (1981)
		Oyster (*Crassostrea gigas*)						5.3 ppb (EC_{50})		
	$CuCl_2$	Oyster (*C. virginica*)	Larvae	25	12 days	55.7 ppb	Single dose	95% mortality	Seawater, static, water changed daily.	Calabrese et al (1977)
		Clam (*M. mercenaria*)			8-10 days	28.0 ppb		95% mortality		
	$CuCl_2$	Mussel (*Mytilus edulis*)	Adult (46-47mm)	18	35 days	20 ppb	Dosing as required	1.4 ppm (ww)	Seawater, static, water changed every 2 days.	Phillips (1976a)
						40 ppb		7.4 ppm (ww)		
	unknown	Mussel (*Mytilus edulis*)	Adult (~46mm)	Aug/Sep		11.1 kg/d	Discharge	0.98 ppm (ww)	From polluted harbour	Phillips (1976b)
	$CuCl_2$	Mussel (*Mytilus edulis*)	Adult (50-80 mm)	13	21 days	25 ppb	Continuous	34.6 ppm (dw)	Samples were total soft tissues, seawater, flow-through	Harrison et al (1983)
						75 ppb		34.5 ppm (dw)		
	$CuCl_2$	Mussel (*Mytilus edulis*)	Adult (4-5 mm)	2.6 - 24	1 year	5 ppb	Continuous	17.9 ppm (ww)	10 ppb toxic after 21 months	Calabrese et al (1984)
					1.5 years	5 ppb		34.9 ppm (ww)	Samples were total soft tissues, seawater, flow-through	
					1.75 yrs	5 ppb		19.2 ppm (ww)		
	$CuSO_4$	Mussel (*Mytilus edulis*)	Adult (4-5 mm)	18	14 days	50 ppb	Single dose	55% mortality	Seawater, static, dose is initial concentration	Maung Myint & Tyler (1982)
				0	70 days	50 ppb		0% mortality		
	$CuSO_4$	Mussel (*Mytilus edulis*)	Adult (10-20 mm)	10	7 days	200 ppb	Single dose	55% mortality	Seawater, static, threshold toxicity 100-200 ppb	Scott & Major (1972)
					7 days	100 ppb		5% mortality		
	$CuSO_4$	Mussel (*Mytilus edulis*)	Adult (30-60 mm)	15	255 hours	90 ppb	Continuous	50% mortality	Seawater, flow-through,	Davenport & Manley (1978)
Lead	$Pb(NO_3)_2$	Mussel (*Mytilus edulis*)	Embryos	17	48 hours		Single dose	476 ppb (EC_{50})	Filtered seawater, static	Martin et al (1981)
		Oyster (*Crassostrea gigas*)						758 ppb (EC_{50})		
	$Pb(NO_3)_2$	Mussel (*Mytilus edulis*)	Adult (46-47mm)	18	35 days	20 ppb	Dosing as required	15.3 ppm (ww)	Seawater, static, water changed every 2 days.	Phillips (1976a)
						40 ppb		20.1 ppm (ww)		
	unknown	Mussel (*Mytilus edulis*)	Adult (~46mm)	Aug/Sep		8.2 kg/d	Discharge	2.37 ppm (ww)	From polluted harbour	Phillips (1976b)
	$Pb(NO_3)_2$	Mussel (*M. galloprovincialis*)	Adult	21	46 days	100 ppb	Dosing as required	40 ppm (ww)	Artificial seawater, static, estimated lethal [Pb]>200 ppb	Majori & Petronio (1974)
Manganese	$MnCl_2$	Clam (*Mya arenaria*)	Adult (5g av meat wt)	16-22	14 days	1440 ppb	Continuous	738 ppm (dw)	Control was 61 ppm (dw), seawater, flow-through	Eisler (1977)

Table 4b: Nonoxidizing Agents- Metals (con't)

Metal	Salt Used	Species	Age	Temp. (°C)	Duration	Conc.	Addition Method	Results	Notes	Author
Mercury	$HgCl_2$	Mussel (*Mytilus edulis*) Oyster (*Crassostrea gigas*)	Embryos	17	48 hours		Single dose	5.8 ppb (EC_{50}) 6.7 ppb (EC_{50})	Filtered seawater, static	Martin et al (1981)
	$HgCl_2$	Oyster (*C. virginica*) Clam (*M. mercenaria*)	Larvae	25	12 days 8-10 days	20.7 ppb 25.4 ppb	Single dose	95% mortality 95% mortality	Seawater, static, water changed daily.	Calabrese et al (1977)
	$HgNO_3$	Mussel (*Mytilus edulis*)	Adult	10	21 days	1 ppb	Single dose	4.2 ppm (ww) gills	Seawater, static, changed daily, control 0.13 ppm (ww) gills	King & Davies (1987)
Nickel	$NiSO_4$	Mussel (*Mytilus edulis*) Oyster (*Crassostrea gigas*)	Embryos	17	48 hours		Single dose	891 ppb (EC_{50}) 349 ppb (EC_{50})	Filtered seawater, static	Martin et al (1981)
	$NiCl_2$	Oyster (*C. virginica*) Clam (*M. mercenaria*)	Larvae	25	12 days 8-10 days	2500 ppb 10300 ppb	Single dose	95% mortality 95% mortality	Seawater, static, water changed daily.	Calabrese et al (1977)
	$NiCl_2$	Clam (*Mya arenaria*)	Adult (5g av meat wt)	16-22	14 days	10 ppb	Continuous	6.7 ppm (dw)	Control was 2.3 ppm (dw), seawater, flow-through	Eisler (1977)
Potassium	KI KCl	Mussel (*D. polymorpha*)	Adult (15-20mm)	20	24 hours		Single dose	226 ppm (LC_{50}) 138 ppm (LC_{50})	Freshwater, static	Fisher et al (1991)
	K_2CO_3	Mussel (*D. polymorpha*)	Adult	Nov	4 days	100 mg/L	Continuous	91-100% mortality	Freshwater, flow-through	Lewis et al (1997)
Selenium		Mussel (*Mytilus edulis*) Oyster (*Crassostrea gigas*)	Embryos	17	48 hours		Single dose	>10000 ppb (EC_{50}) >10000 ppb (EC_{50})	Filtered seawater, static	Martin et al (1981)
Silver	$AgNO_3$	Mussel (*Mytilus edulis*) Oyster (*Crassostrea gigas*)	Embryos	17	48 hours		Single dose	14 ppb (EC_{50}) 22 ppb (EC_{50})	Filtered seawater, static	Martin et al (1981)
	$AgNO_3$	Oyster (*C. virginica*) Clam (*M. mercenaria*)	Larvae	25	12 days 8-10 days	35.7 ppb 46.2 ppb	Single dose	95% mortality 95% mortality	Seawater, static, water changed daily.	Calabrese et al (1977)
	$AgNO_3$	Mussel (*Mytilus edulis*)	Adult (4-5 mm)	2.6 - 24	6 months 1 year	50 ppb 50 ppb	Continuous	1.77 ppm (ww) 2.99 ppm (ww)	10 ppb toxic after 21 months Samples were total soft tissues, seawater, flow-through	Calabrese et al (1984)
Zinc	$ZnSO_4$	Mussel (*Mytilus edulis*) Oyster (*Crassostrea gigas*)	Embryos	17	48 hours		Single dose	175 ppb (EC_{50}) 119 ppb (EC_{50})	Filtered seawater, static	Martin et al (1981)
	$ZnCl_2$	Clam (*M. mercenaria*)	Larvae	25	8-10 days	341 ppb	Single dose	95% mortality	Seawater, static, water changed daily.	Calabrese et al (1977)
	$ZnCl_2$	Clam (*Mya arenaria*)	Adult (5g av meat wt)	16-22	14 days	500 ppb	Continuous	804 ppm (dw)	Control was 109 ppm (dw), seawater, flow-through	Eisler (1977)
	$ZnSO_4$	Mussel (*Mytilus edulis*)	Adult (4-5 mm)	18	98 days	200 ppb	Single dose	chronically inhibited gamete maturation	Seawater, static, dose is initial concentration	Maung Myint & Tyler (1982)
	$ZnCl_2$	Mussel (*Mytilus edulis*)	Adult (46-47mm)	18	35 days	100 ppb 400 ppb	Dosing as required	51.9 ppm (ww) 73.7 ppm (ww)	Seawater, static, water changed every 2 days.	Phillips (1976a)
	unknown	Mussel (*Mytilus edulis*)	Adult (~46mm)	Aug/Sep		19.9 kg/d	Discharge	97.1 ppm (ww)	From polluted harbour	Phillips (1976b)

CF[1]: concentration factor

EC_{50}[2]: concentration which caused 50% abnormal development in larvae.

ppb: parts per billion ~ µg/L ~ µg/kg

ppm: parts per million ~ mg/L ~ mg/kg

effects on zinc accumulation in the mussel. Results showed that zinc accumulation varies seasonally. The zinc uptake in mussels is unaffected by low temperatures and salinities. The presence of other metals did not noticeably influence zinc accumulation. A follow-up study (Phillips, 1976b), used Zn levels (soft-tissue) in mussels from the field, to determine if they reflect elevated zinc levels from known discharge sites. The author reported a very good agreement between mussel zinc concentrations and known discharge levels, with concentrations decreasing proportionally to the distance from the source.

The effects of zinc ($ZnSO_4$) exposure and accumulation on the reproductive process in the mussel (*Mytilus edulis*) was explored in a U.K. study (Maung Myint & Tyler, 1982). Adult (43-46mm) mussels were exposed to 200 ppb Zn, in static seawater, at various temperatures for more than 4 months. The authors concluded that zinc was less toxic than copper, and had negative effects on reproduction. Zinc seemed to inhibit maturation of gametes and destroyed developing gametes. The authors implied, that results were influenced by the probability that ionic zinc is more toxic than the organically-bound zinc, which predominates in the marine environment.

Stromgren (1982) investigated the effect on growth rate in mussels (*Mytilus edulis*) by exposing them to elevated (10-200 ppb) zinc levels, over a period of 10-22 days. The adult mussels (19-29mm) were placed in a flow-through seawater system, at 17°C, dosed with zinc ($ZnCl_2$). Growth rate was significantly affected at concentrations as low as 25 ppb Zn, but the author estimated that 60 ppb Zn would be required to inhibit growth by 50%. When exposed to 200 ppb zinc, the treated mussels showed no visible change in behavior compared to the controls.

These studies indicate that a zinc salt would not be an effective treatment for controlling mussel infestation because toxicity is too low and not specific to the mussel. Zinc could play a role in biofouling control in the future, but not without further research.

MOLECULAR REGULATORS

Attempts to solve the very complex problem of biofouling has produced some novel ideas. Recent research has led to a much greater understanding of the interaction between the different stages of biofouling. It is assumed that chemical interactions between the different stages of biofouling exist (and act as signals) (Kirchman & Mitchell, 1981, 1983; Holmstrom & Kjelleberg, 1994). Several researchers suggested that biofouling control may be achieved by interruption or manipulation of these chemical signals.

Manipulation of chemical signals was illustrated by some marine research applied to several organisms representing different phyla. These studies identified the chemical or molecular regulators for larval settlement. The idea was to chemically manipulate this

process, thereby interfering with the settlement and metamorphosis of the larval stage. A compound that had no toxic effects, instead just conveyed chemically, a signal that discouraged settlement sounded promising. This idea was compromised by the discovery of a potential serious problem with this approach: the same molecular regulators for larval settlement are closely related to regulators of neuronal and developmental processes in many animals, including mammals (Morse, 1994). The author concluded that the implications were too great to proceed with this approach. But he suggests that control of molecular regulators of other processes be investigated (i.e. byssal attachment).

Some freshwater studies, involving the use of serotonin to manipulate the reproductive cycle of the zebra mussel have been reported. The trials proved that serotonin affected spawning behavior in the mussels (Ram & Nichols, 1990). This could prove useful for power plants that use natural cooling water by predicting or controlling local mussel spawning habits. The one drawback is the lack of information regarding the effects on non-target organisms. Some of the possible effects include disruption of the reproductive cycle and the impairment of proper growth and development.

This approach holds some real promise, although, it is still in the research stage. Years could pass before the proper chemical (that will interrupt or control a necessary process in biofouling organisms) is determined. An appropriate compound may never be found that will be effective in controlling biofouling, while being environmentally acceptable.

NATURAL PRODUCTS & ANALOGS (Table 4c)

A number of years ago, researchers noted that some sessile marine organisms (i.e. octocorals, sponges) remain virtually free of biofouling. Research proceeded to discover the mechanism(s) that provide this protection. Some organisms avoided the biofouling problem by regularly sloughing off their external covering, exposing a fresh, clean surface. It was believed that others combatted biofouling by means of some chemical defense.

Extracts from these organisms were collected to investigate the chemical composition and test for any antifouling properties. This led to the next problem, sample quantity. How were they going to test such a small quantity of extract for antifouling properties? Since so much of this work was being done in Japan, it was no surprise that a Japanese group developed a technique for assessing these compounds (Harada et al., 1984). Further work led to improvements of this initial technique (Ina et al., 1989; Takasawa et al., 1992; Kitajima et al., 1995; Hayashi & Miki, 1996) and work continues on assessment methods. The method that has seen the most use for assessing these compounds, is a technique proposed by Ina et al. (1989). This method involves coating a

small surface with the compound and then placing a mussel on the treated surface and observing the response. The response is gauged by mussel movement and the attachment of byssal threads. To rate the level of settlement inhibition of the test compound, copper sulphate ($CuSO_4$) is used as the control. Another procedure used the retraction of the mussel foot as a good indication of repellent activity.

Most researchers presume that eventually these compounds will be used as paint additives, for antifouling purposes. These natural compounds are in limited supply and attempts to synthesize these and similar compounds in the laboratory, have had limited success. Many of these compounds are so chemically complex that synthesis is proving very difficult. Table 4c contains a more detailed list of organisms and compounds being examined.

One of the first compounds to be considered was from endod (*Phytolacca dodecandra*), a plant found in Ethiopia and other African countries. The dried, crushed berries are used as a laundry detergent, which seemed to have lethal effects on local snails. Attempts to use endod as a means of controlling zebra mussel infestations (Lee et al., 1993; Mezui & Lee, 1993), indicate that endod was toxic to the zebra mussel at concentrations of 20 ppm and higher. At lower concentrations of endod, the byssal attachment of the zebra mussel seemed to be weakened or inhibited. Authors concluded that the endod did not attack the byssal thread chemically, instead it seemed to affect the byssal gland itself. Unfortunately, there were no seawater applications described in the literature.

Based on the literature available, the most researched group of organisms are the octocorals. A variety of compounds have been extracted from many different species for antifouling studies. The following is a partial list of some of the species examined and the source of the publication: sea pansies, *Renilla reniformis* (Rittschof et al., 1986); gorgonian octocoral, *Leptogorgia virgulata* (Gerhart et al., 1988); octocoral, *Dendronephthya* sp. (Mizobuchi et al., 1993; Kawamata et al., 1994); octocoral *Sinularia* sp. (Mizobuchi et al., 1994, 1996). Many of the compounds isolated from these octocorals showed promise as mussel repellents at surprisingly low concentrations. Unfortunately, these were all laboratory studies performed under very controlled conditions, not long-term field trials.

Marine sponges have also acquired considerable attention as potential sources of naturally produced antifouling compounds. Some of the other marine organisms investigated for antifouling properties are: algae, ascidians, bacteria, bryozoa and even eelgrass. The list continues to grow in this very active field of research.

The probability of one of these compounds finding a commercial application is still years away, due to existing problems (i.e. supply, cost). Then there are application problems, such as, if paint incorporation is considered, will the paint affect the active properties of the compound? How quickly will the compound leach out of the paint?

Table 4c: Nonoxidizing Agents- Natural Products and Analogs

Source Species	Chemical Description	Extraction Method	Fouling Organism Tested	Test Medium	Conc. Tested	Result	Author
Sea Pansies (*Renilla reniformis*)	low molecular wt. substances	methanol methylene chloride	Barnacle larvae (*Balanus amphitrite*)	22h settlement test,seawater in polystyrene container	1 - 30 mg/ml 3 - 20 mg/ml	100% settlement inhibition 90% settlement inhibition	Rittschof et al (1986)
Octocoral (*Leptogorgia virgulata*) Gastropod (*Neosimnia uniplicata*)	diterpenes	methanol/ methylene chloride	Barnacle larvae (*Balanus amphitrite*)	24h, 28°C, settlement test, seawater in glass vials	various	EC_{50}[1]: 5µg/ml settlement inhib. EC_{50}: 4µg/ml settlement inhib.	Gerhart et al (1988)
Brown algae (*Costaria costata*) (*Undaria pinnatifida*)	glycerols	methanol/benzene	Mussel (adult) (*Mytilus edulis*)	Sample on 4 cm(dia.) special cardboard in running seawater	4.0 mg/cm^2 4.0 mg/cm^2	(++) repellent activity[2] (++) repellent activity	Katsuoka et al (1990)
Sponge (*Lissodendoryx isodictyalis*)	terpenoids	ethyl acetate/ methylene chloride	Barnacle larvae (*Balanus amphitrite*)	22h settlement test,seawater in polystyrene container	10 ng/ml >400 µg/ml	significantly inhibited settlement killed all larvae	Sears et al (1990)
Eelgrass (*Zostera marina* L.)	phenolic acid sulphate ester	methanol/hexanes	marine bacteria	Glass slide, 4h, 50 ml seawater	various	EC_{50}[3]: ~10µg/cm^2 bac. density	Todd et al (1993)
Octocoral (*Dendronephthya* sp.)	sterols/fatty acid mixture	ethyl acetate/acetone	Mussel (adult) (*Mytilus edulis*)	Sample on 4 cm(dia.) special cardboard in running seawater	0.4 mg/cm^2	(++) repellent activity	Mizobuchi et al (1993)
Sponge (*Phyllospongia papyracea*)	fatty acids	methanol/ carbon tetrachloride	Barnacle larvae (*Balanus amphitrite*)	Seawater in coated petri dish, 2 days at 25° C	20 µg/cm^2 10 µg/cm^2	100% settlement inhibition 13% settlement inhibition	Golo et al (1993)
Endod (*Phytolacca dodecandra*)	saponins	not given	Mussel (adult) (*D. polymorpha*)	Freshwater study	≤ 20 mg/L	Lethal, or weaken, inhibit attach. Target site- byssal gland	Mezui & Lee (1993)
Octocoral (*Sinularia* sp.)	compound A[4] compound B[5]	ethyl acetate/acetone	Mussel (adult) (*Mytilus edulis*)	Sample on 4 cm(dia.) special cardboard in running seawater	0.4 mg/cm^2 0.2 mg/cm^2	A : (+) repellent activity B : (++) repellent activity	Mizobuchi et al (1994)
Octocoral (*Dendronephthya* sp.)	trigoneliine ($C_7H_7NO_2$)	ethanol/ ethyl acetate	Barnacle larvae (*Balanus amphitrite*)	Seawater in coated petri dish, 1 days at 25° C	2-10 ppm > 20 ppm	Min. conc. to inhibit settlement Conc. causes 50% mortality	Kawamata et al (1994)
Bryozoan (*Zoobotryon pellucidum*)	2,5,6-tribromo-1-methyl-gramine	methanol/acetone	Mussel (adult) (*Mytilus edulis*)	Sample on 4 cm(dia.) special cardboard in running seawater	0.03 µg/cm^2	(++) repellent activity with relatively low toxicity	Kon-ya et al (1994)
Octocoral	compounds C[6] and D[7]	not given	Diatom (*Nitzschia* spp.)	Continuous release system to mimic an octocoral	10-50 µg/cm^2/day	More successful at inhibition of diatom fouling than $CuSO_4$	Targett & Stochaj (1994)
Ascidian (*Claveilina lepadiformis*)	not given	filtered seawater	Brine shrimp nauplii (*Artemia* sp.) Bryozoan larvae (*F. hispida*) Hydroid larvae (*Tubularia larynx*)	Static, seawater at 17° C for 24 hrs	0.05 g wet wt/ml[8] 0.01 g wet wt/ml 0.05 g wet wt/ml	98% mortality 100% mortality 100% mortality	Teo & Ryland (1995)
Endod (*Phytolacca dodecandra*)	saponins	powder (crushed berries)	Mussel (adult) (*D. polymorpha*)	Static, freshwater, at room temperature for 24 hrs.	8.8 ppm 20.0 ppm	50% mortality 90% mortality	Lee et al (1993)
Macroalgae (9 species) Marine invertebrate (3 species)	not given	ethanol/ methylene chloride	Mussel (adult) (*Mytilus edulis*)	Sample on (4x4) cm slate, in running seawater for 65 hrs.	10 ml extract dried on slate	(++) repellent activity for 7 organisms extracted	Vanelle & Le Gal (1995)
Octocoral (*Sinularia* sp.)	$C_{20}H_{30}O_4$	acetone then water/ dichloromethane	Mussel (adult) (*Mytilus edulis*)	Sample on 4 cm(dia.) special cardboard in running seawater	0.7 mg/cm^2	(++) repellent activity	Mizobuchi et al (1996)
Sponge (*Crella incrustans*)	*lyso*-PAF[9]	acetone/ 1-butanol/ hexanes/ methanol	Black mussel (*Xenostrobus pulex*)	Sample on (7x13)cm cardboard, static, seawater, 18°C,overnight	4.4 µg/cm^2	significant inhibition of byssal attachment	Buller et al (1996)
Eelgrass (*Zostera marina* L.)	*p*-(sulphooxy) cinnamic acid	synthetic powder	Barnacle Bryozoa	Continuous release system to mimic an octocoral	500 µg/cm^2/day	50% reduction of fouling 50% reduction of fouling	Hasbeck et al (1996)

EC_{50}[1]: concentration which caused 50% settlement inhibition

(++) repellent activity[2]: from Ina et al (1989), based on comparison to activity of $CuSO_4$

EC_{50}[3]: concentration which reduces bacteria density by 50%

compound A[4]: 13- acetoxypukalide

compound B[5]: (9E)-4-(8,10-dimethylocta-9,11-dienyl) furan-2-carboxylic acid

compound C[6]: 3-acetyl-2,5- dimethyl furan

compound D[7]: 2-furly-n-pentyl ketone

g wet wt/ml[8]: weight of macerated frozen material per ml seawater

lyso-PAF[9]: 1-hexyldecyl-sn-glycerol-3-phosphorylcholine

What is the acceptable leach rate required for adequate biofouling control? These questions require answers prior to any commercial applications.

PAINT ADDITIVES: ORGANOMETALLICS (Table 4d)

Organotin is very effective as a hull paint additive for controlling biofouling. Laboratory mortality tests support this fact; organotin is very toxic at low levels to the mussel (*Mytilus edulis*) larvae (Beaumont & Budd, 1984) and juvenile adults (Stromgren & Bongard, 1987). For this reason, organotin is used as the reference in evaluating new paint additives. Organotins are available in a number of different forms, but the active ingredient in all the forms is organically-bound tin (Table 4d). However, the toxicity of organotin to non-target organisms, and its resistance to biological degradation (Lee, 1989), has resulted in a ban on future use in antifouling paints, except in special circumstances. So far, no other organometallic paint additive tested, has been nearly as effective as organotin.

PAINT ADDITIVES: OTHERS (Table 4e)

Metal oxides (i.e. copper, tin, zinc) have been tested for the partial replacement of organotin as paint additives. They all proved rather ineffective alone, but several showed promise when tested in combination. Cuprous and zinc oxide, in combination with organotin, seemed quite effective compared with the organotin alone (French & Evans, 1986), in a short-term panel test (not an actual ship trial). Cuprous and stannous oxide also showed some potential, although this 48- hour laboratory study considered only one fouling species (Personne & Castritsi-Catharios, 1989).

The number of organic-based compounds being considered and tested is enormous, and continues to grow. A number of commercial compounds show promise as paint additives, but many of these compounds would be unacceptable for direct use. Sea-Nine™211 (Rohm & Haas Company, USA), Diuron (Bayer, UK) and Chlorothalonil (ISK Biotech, USA) all proved to be somewhat effective. There is concern over their use due to non-target organism and environmental effects. Also, Sea-Nine™211 degrades too quickly in seawater, while diuron seems to be non-biodegradable (Callow & Willingham, 1996). Irgarol®1051 (Ciba-Geigy, Switzerland) has seen some freshwater applications, but there is also concern about its resistance to biodegradation. Little is known about its effect on non-target marine organisms and the environment (Pearce, 1995; Toth et al., 1996). Some quaternary ammonium salts have been tested and show some promising results, but are unstable in seawater. The same applies for those antibiotics that have undergone preliminary tests.

Table 4d: Nonoxidizing Agents- Paint Additives: Organometallics

Compound Tested	Type of Fouling	Concentration / Release Rate	Results	Source
trialkyltin / triaryltin	general marine fouling	not given (30 mole % TBTM[1])	0% fouling for 42 months using 3 diff. film depths (5,11,13 mils) on ship hull	Fischer (1982)
tributyltin oxide (TBTO)	Mussel larvae (veliger) (*Mytilus edulis*)	5 d in seawater at 10 µg/L 10 d in seawater at 1 µg/L	Mortality test, TBT in solution with test organisms	Beaumont and Budd (1984)
tributyltin chloride (TBTCl)	general marine fouling	not given (14% w/w TBTCl)	0% fouling for 49 months using 3 coats on test panels in seawater in Florida	Porter & Miale (1984)
tributyltin (TBT)	Oyster spat (*Crassostrea gigas*)	49 d in seawater at 20-200 ng/L	Observed shell thickening that was proportionally related to TBT concentration	Thain et al (1987)
dibutyltin (DBT)	Oyster spat (*Crassostrea gigas*)	49 d in seawater at 82-110 µg/L	50% mortality	
tributyltin oxide (TBTO)	Mussel (juvenile 5-8mm) (*Mytilus edulis*)	7 d in seawater at > 0.4 µg/L	Significant toxic effects, increasing with exposure time	Stromgren & Bongard (1987)
tributyltin (TBT)	Mussel (juvenile 12-14mm) (*Mytilus edulis*)	196 d in seawater at 70 ng/L	Produced reduced growth rates	Salazar & Salazar (1987)
tributyltin (TBT)	general marine fouling	not given	Up to 7 yrs of outstanding antifouling performance on US Naval ships	Dowd (1988)
tributyltin chloride (TBTCl)	barnacle fouling hydrozoan fouling	0.22 µg/cm^2/day 0.83 µg/cm^2/day	90% fouling reduction 90% fouling reduction	Halsbeck et al (1990)
organoantimonial substances	general marine fouling	not given, used in combin. with other paint additives	Claimed to be effective for more than 30 months	Anon. (1984)
zinc pyrithione	marine fouling (soft)	not given	Company claims in combination with cuprous oxide, it is an alternative to TBT.	Internet (see ref.)

TBTM[1]: tributyltin methacrylate

Table 4e: Nonoxidizing Agents- Paint Additives: Others

Name of Additive	Test Species	Addition Method	Results & Notes	Source
ametryne[1]	marine biofouling	Test panels in seawater for 3 yr period	Tested in combination with TBT and CuO in various test paints with very promising results.	Bocksteiner et al (1976)
1-dodecylguanidine acetate (1-DGA) 2-dodecylguanidine acetate (2-DGA)	marine bacteria (*A. coffeaeformis*)	Tested in artificial seawater, at 20°C for 96 hrs	Tested in combination with TBTO, both inhibited bac. growth better than TBTO alone. (2-DGA better)	Evans et al (1986)
cuprous oxide zinc oxide	marine biofouling	Copolmer containing TBT, on test panels in field for 15 mon	CuO, ZnO, CuO+ZnO, tested, all performed better than TBT alone, CuO and CuO+ZnO same results	French & Evans (1986)
cuprous oxide	marine biofouling	Ablative copper paint	Tested on US Naval ship hulls, not as effect as TBT copolymer previously used.	Dowd (1988)
cuprous oxide stannous oxide (SnO)	larval brine shrimp (*Artemia*)	Test panels in seawater, at 25°C for 48 h	CuO alone, CuO+SnO were tested, both commercial paints,CuO+SnO gave better mortality results.	Persoone & Castritsi-Catharios(1989)
alkyldimethyloctyl ammonium (quaternary ammonium salts)	marine bacteria	Exp. varnish and paint, PVC sheets in seawater for 4 mon	Better results with varnish, both(v&p) outperformed commercial tin-based paint.	Mellouki et al (1989)
2,4-dinitrophenol (DNP) benzoic acid	release rate of biocide in seawater	Ablative and non-ablative coatings tested.	Poor results for all combinations, DNP hydrolyzed too quickly, benzoic acid release rates too low.	Weisman et al (1992)
chloramphenicol (antibiotic)	marine bacteria (g. Oceanospirillum)	Painted steel panels, artificial seawater at 20°C for 24-48 hrs	Tested in commercial Cu paint, 100% inhibition slime-forming bacteria with chloramphenicol.	Stiffey et al (1992)
tetracycline (antibiotic)	release rate in seawater	Mixed with commercial Cu antifouling paint	Poor results, tetracycline leached too rapidly from paint to be effect long-term antifoulant.	Peterson et al (1993)
Gram-negative bacterium (marine)	barnacle larvae (*B. amphitrite*)	Bacteria immobilized in polyacrylamide gel (hydrogel)	94-100% loss in larval viability, showed good stability to 90d field test in seawater.	Gatenholm et al (1995)
Sea-Nine™ 211[2] diuron[3] chlorothalonil[4] Irgarol® 1051[5]	marine bacteria (*A. coffeaeformis*)	Test panels in seawater, at 25°C for 24h	0.5 mg/L inhibited growth by 80% 0.02 mg/L inhibited growth by 80% 2.0 mg/L inhibited growth by 80% 0.02 mg/L inhibited growth by 80%	Callow & Willingham (1996)
Irgarol® 1051	mysid shrimp oyster larvae (*C. virginica*)	Tested in solution (seawater)	96h at 0.4 mg/L caused 50% mortality 48h at 3.2 mg/L caused 50% mortality	Toth et al (1996)

ametryne[1]: 2-ethylamino-4-isopropylamino-6-methylthio-1,3,5-triazine
Sea-Nine™ 211[2]: (4,5-dichloro-2-n-octyl-4-isothiazolin-3-one)
diuron[3]: (3-(3,4-dichlorophenyl) 1-1-dimethylurea)
chlorothalonil[4]: (2,4,5,6 tetrachloro 1,3 benzendicarbonitrile)
Irgarol® 1051[5]: (2-methylthio-4-tert-butylamino-6-cyclopropylamino-s-triazine)

These compounds suffer complications when incorporated in paint. Some of these problems were mentioned in the previous section. Even if these problems can be overcome, release rate of the antifouling agent must be properly controlled. If the release rate is too quick, the effective lifetime of the paint will be too short. Conversely, if the release rate is too slow, the paint will not control biofouling. Ideally, the release rate should be fast enough to control biofouling, yet slow enough to offer protection for an extended period of time (Haslbeck et al., 1990).

Prior mention was made that a paint would not be a practical solution for controlling internal fouling of cooling water pipes. Some of these additives appear promising if they could be considered in direct applications. Unfortunately, due to present concerns and the lack of knowledge about long-term effects on non-target organisms and the environment, direct application would probably be prohibited.

NONCHEMICAL ANTIFOULANTS

Many of the previously discussed chemical treatments would be considered proven methods, although some have very specialized applications. The basis of their effectiveness is linked to the mortality of the target organism(s). For many, this toxicity is also the reason for increasing concern. As a result of the lack of discrimination between target and non-target organisms, coupled with environmental impact, strict regulations regarding use of many of these chemicals in antifouling applications have been implemented.

The one obvious exception, is the group of naturally occurring compounds. Many of these compounds are designed to repel or discourage settlement rather than kill. This eliminates concern for non-target organisms and since they are natural products, generally they have no environmental impact (at levels found naturally in the environment). Most of the research at present, concentrates on non-toxic methods of control, that is, the aim is generally to repel or discourage settlement. Even those that cause mortality, have the advantage of little or no environmental impact. With present chemical contamination problems, the regulations over use and discharge of chemicals will get stricter. Therefore, in the long-term, the more feasible choice of biofouling control methods, would be non-toxic in nature.

THERMAL (Table 5)

This method of control will result in mussel mortality, but the long-term environmental effects are minimal compared with chemical methods. There is a noticeable lack of values for minimum temperature tolerance for marine mussels (Table 5). The reason for this is that blue mussels (*Mytilus edulis*) have a very good tolerance to freezing; they have been known to survive months frozen in ice in northern climates. Reduced growth rate seems to be the only effect on the survival of the mussel in colder climates. It appears that increased temperature is the only thermal method likely to control blue mussel infestation.

Before considering thermal tolerance of the mussel, we should consider what effects elevated temperatures have on the mussel. Oxygen consumption is not drastically affected, but elevated temperatures cause an increase in the rate of metabolism in the blue mussel (Widdows, 1973), including filtering and feeding rates. If temperatures begin to approach lethal levels, the mussel responds by closing up and isolating itself from its surroundings, although physiological requirements eventually force the mussel to reopen. A study at a power plant investigated the effect on blue mussels exposed to heated cooling water. Mussels can survive intermittent exposure (via valve closure) to heated seawater provided the exposure time is not too extended (Gonzalez & Yevich, 1976).

Mussels that inhabit warmer waters are more tolerant to thermal control, and require higher temperatures to induce mortality. Mussels from a northern climate are less tolerant to elevated water temperatures. Greater tolerance was observed when mussels were given time to acclimatize to a gradual elevation in temperature compared to control mussels. This phenomenon applies to both the larval (Wright et al., 1983) and adult (Wallis, 1975; Stock & Strachan, 1977) stage of the marine mussel. Tolerance to elevated temperatures seems to be species dependent. The tolerance of two local marine mussel species (of the same size) to elevated temperatures was studied, and at all temperatures tested, *Mytilus edulis* required a longer exposure time than *Mytilus californianus* to achieve 100% mortality (Fox & Corcoran, 1957). While another local species, *Mytilus Hormomya adamsianus*, was even more tolerant to elevated temperatures than *M. edulis* was.

Size also influences temperature tolerance of adult marine mussels. Large mussels are more tolerant to elevated water temperatures (Wallis, 1975). In a study of the green mussel (*Perna indica*), Rajagopal et al. (1995a,b), tested a wider size range. Therefore, in control of mussel infestations, size will have to be taken into account as it influences the temperature and exposure requirements.

The thermal method is currently being used for controlling invertebrate infestation in a number of power plants around the world (Jenner, 1983; Khalanski, 1993). It offers many advantages over chemical treatment provided the cooling water system is appropriately designed. Some of the advantages are cost (heat is a waste product), minimal environmental effects, plus it would not require any special handling or safety

Table 5: Thermal Methods

Species Tested	Location	Age/Size	Temperature/Conditions	Reference
Mussel (*Mytilus californianus*)	California	juv.adult (1-3mm)	40.9°C for 40 min - 100% mortality 37.8°C for 50 min - 100% mortality 35.0°C for 6 hrs - 100% mortality 32.2°C for 12 hrs - 100% mortality	Fox & Corcoran (1957)
Mussel (*Mytilus edulis*)	California	juv.adult (1-3mm)	40.9°C for 50 min - 100% mortality 37.8°C for 60 min - 100% mortality 35.0°C for 7 hrs - 100% mortality 32.2°C for 14 hrs - 100% mortality	
Mussel (*Mytilus edulis*)	Connecticut	larvae	30°C - 0.3% survival (25‰ salinity) 25°C - 60.1% survival (25‰ salinity) 20°C - 79.3% survival (25‰ salinity)	Brenko & Calabrese (1969)
Mussel (*Mytilus edulis*)	Australia	adult (30-50mm)	26.8°C - ILT[1] (acclimatized 15d at 21°C) 28.2°C - ILT (acclimatized 25d at 21°C)	Wallis (1975)
Mussel (*Mytilus edulis*)	Massachusetts	adult	27 - 28°C lethal temperature, observed impaired feeding at 25°C.	Gonzalez & Yevich (1976)
Mussel (*Mytilus edulis*)	California	adult	97°F for 3.6 hrs - 100% mortality 101°F for 1.2 hrs - 100% mortality 105°F for 0.41 hrs - 100% mortality	Graham et al (1977)
Mussel (*Mytilus edulis*)	San Diego Bay	adult (5-30 and 40-70mm)	90°F for 22.3 hrs - 95% mortality 97.5°F for 2.7 hrs - 95% mortality 105°F for 0.2 hrs - 95% mortality	Stock & Strachan (1977)
Mussel (*Perna viridis*)	India	adult (2.3mm) adult (29.7mm) adult (69.6mm) adult (110.6mm)	41°C for 24 min - 100% mortality 41°C for 79 min - 100% mortality 41°C for 87 min - 100% mortality 41°C for 85 min - 100% mortality	Rajagopal et al (1995a)
Mussel (*Perna indica*)	India	adult (8.9mm) adult (18.3mm) adult (25.5mm) adult (36.0mm)	41°C for 26 min - 100% mortality 41°C for 44 min - 100% mortality 41°C for 54 min - 100% mortality 41°C for 63 min - 100% mortality	Rajagopal et al (1995b)

ILT[1] : incipient lethal temperature

concerns of many chemical applications. Adapting an existing cooling water system to accommodate thermal back-flushing, would be quite costly. The method requires the system to tolerate a certain level of infestation that occurs between treatments, along with the problem associated with the disposal of dead mussels.

NONTHERMAL ENERGY

ACOUSTIC ENERGY

Attempts to control mussel settlement through applications of acoustic energy date back more than a decade. A study done in the Netherlands (Jenner, 1983), investigated the effects of acoustic energy by: (1) using sound or shock waves from small explosions detonated in the vicinity of settling mussels, and by (2) using electromechanically generated acoustic waves at frequencies from 20 Hz to 200 kHz. Attempts proved ineffective: no reaction was observed.

Branscomb & Rittschof (1984), examined the effects of low frequency (~ 30 Hz) sound waves and other factors on barnacle settlement. Low frequency sound negatively affected both barnacle settlement and larval metamorphosis, but no long-term deleterious effects were reported. In a study of zebra mussels (*Dreissena polymorpha*), Menezes (1991) discovered that high intensity acoustic energy disintegrated mussel veligers, shattered (shells) juvenile zebra mussels, and killed adult mussels subjected to a sufficient exposure time. Kowalewski et al. (1993), tested the effectiveness of using solidborne acoustic energy (3-18 kHz) to deter attachment of zebra mussels at a water handling facility. Effective prevention of mussel attachment was observed at sonic frequencies in the range of 8-14 kHz. Such acoustic energy did not result in structural damage to the water handling system.

No indication was given about the effective range of this method. Would a large number of acoustic units be required for an extensive piping system, if so, to what extent would that increase costs? The noise associated with such a method could also prove to be troublesome.

ELECTRICAL

The use of electrical methods to control biofouling is relatively new. Preliminary work began about ten or fifteen years ago, with the investigation of low voltage electric

fields, at various intensities, by way of a 60 Hz alternating current (AC), continuous direct current (DC) and pulsed DC (Smythe et al., 1991). Results were encouraging, but the technique was unable to achieve 100% control of mussel settlement. Essentially, when a current is passed between electrodes immersed in water, a field is generated in the water. The field spreads out from the anode and forms an even field distribution or density between the anode and cathode, and in theory, the electric field could stun (not kill) mussels passing through, and thereby, discourage settlement.

Pulse power (the relatively rapid rising 'pulse' shaped signal produced by capacitor discharge) generated electric fields were also employed to control zebra mussel infestation (Smythe et al., 1995). A commercially available fish barrier pulsator (set at 100 Hz with a pulse width of 0.5 milliseconds and field strength measured at 15.75 volts/cm^2) produced square wave signals. In theory, mussels are exposed to between 40 and 80 pulses while passing between the anode and cathode. With this set-up, zebra mussel settlement was reduced by 78 to 88%, however, the mussels recovered quite rapidly from the stun effects of the 'pulsed' electric field. In addition, mussels were more sensitive to a unidirectional (DC) fast rising (pulse power) signal, than to a 60 Hz, AC signal. Some design changes would be required to rectify a plating (calcium carbonate) problem on the cathode.

Effectiveness of pulsed electric fields in the control of fouling organisms in tidal waters was also investigated (Schoenbach et al., 1997). The test area was a PVC pipe measuring 4.5 m in length and 1.5 cm inner diameter. Original tests which applied a pulsed field amplitude of 12 kV/cm and a duration of 0.7 μs with a repetition rate of 12 Hz, completely controlled biofouling for a period of 20 days. A second test with the same conditions but a reduced electric field (6.45 kV/cm), produced the same level of control for a period of 23 days. Increasing pulse duration, from 0.7 μs to 4 μs, decreased efficiency from about 100 to 50%. For a 99% efficiency in tidal water with a resistivity of 50 ohm · cm, electrical fields of about 3 kV/cm were required, at a pulse duration of 0.77 μs. Electrical costs under such conditions were 26,000 litres treated water/kWh, or about ten cents. Aquatic species were less affected by pulsed electric fields at lower water temperatures.

A product that was discovered during an Internet search, the Zeta Rod™ (http://zetacorp.com/tourzetarod.html), is a ceramic electrode that also disinfects through use of an electric field. The manufacturer claims the device is very resistant and effective in preventing scale, corrosion and biofouling. Requiring a primary field, with potentials above 5 kV, to achieve effectiveness extending about 20 feet in either direction from the electrode. To benefit from such a method, an elaborate cooling water system would require the installation of many rods throughout the system, which could be problematic and costly.

The pulse power technique looks very promising, but many questions remain. How does water temperature affect efficiency? How long does the "stun"-effect last? Tests were performed over very short distances of piping. Considering an extensive pipe

system, would it require numerous installations along the piping to maintain efficiency? If so, costs would increase. From the above mentioned study (Schoenbach et al., 1997), the electrical costs would be reasonable, but the expense of installing one or more pulse power systems could prove quite costly, especially if repairs and maintenance is frequently required.

ELECTROCHEMICAL

The commercially available "Cathelco" system, a cathodic protection system, has been marketed for decades to combat corrosion and antifouling in marine pipework. The operation of the system involves the dissolution, by way of an electrical current, of two anodes (one copper, one aluminum) in the cooling seawater. The copper anode releases copper ions and the aluminum anode releases aluminum which reacts with water to form gelatinous aluminum hydroxide. The aluminum hydroxide traps the copper ions while migrating to the cathode (internal wall of the pipework). This coating or protective layer of copper, ultimately, offers protection against larval invertebrate settlement (especially mussel larvae). In essence, copper is the biofouling agent, and the gelatinous aluminum hydroxide binds the copper to the wall of the piping (Anon., 1977a; Anon., 1977b).

Lewis (1980), used a flow-through, seawater experiment to evaluate the effectiveness of the "Cathelco" system in controlling biofouling. The system was operated as specified by the manufacturer, for more than 60 days. Based on the loss in weight of the copper anode and the flow rate, seawater copper concentrations should have increased by 2.8 ppb. The results showed a gradual increase in biofouling with time, and the author concluded, that under the specific test conditions employed, the system was not effective in controlling macrofouling.

More recently, attempts have been made to design a system similar to the 'Cathelco' system in principle, to offer protection against fouling and corrosion. This system is intended to protect ferrous materials, and is designed for use on the hull of a ship (Salvago et al., 1991). The system provided an improved level of protection, but there were a few problems regarding practical applications and environmental effects.

Current interest in electrochemical control of biofouling utilizes a different electrochemical method. The earliest proposed method (Nakasono et al., 1993) involved the application of a carbon-chloroprene sheet to the surface to be protected. This sheet is composed of carbon black and graphite, the electrically conductive material, bound in chloroprene rubber. Passing a current through this electrode controls microfouling through the electrochemical oxidation of intercellular coenzyme A contained in the cells of the microfoulers. This is different from electrolysis of seawater which produces toxic oxidizing (oxidants) substances. Results showed it was very effective in controlling bacterial growth at applied potentials of -0.6 to 1.2 V versus the saturated calomel

electrode (SCE). Tests on mussels (*Mytilus edulis*) under these conditions, did not inhibit attachment by byssal threads. However, the authors maintain, that if the microfouling is eliminated it will also control macrofouling due to the relationship between the two stages of biofouling. The author did indicate that after one year of immersion, the sheet began to develop cracks and the electrical conductivity was reduced.

Okochi et al. (1995) employed the same antifouling principle, but different products. His control method was also directed at microfouling by the same electrochemical oxidation of intercellular coenzyme A, illustrated by immersing a bacterial coated electrode in seawater containing a small (0.25 mM) amount of ferrocene monocarboxylic acid (FCA). An applied potential of 0.6 V versus SCE, in 0.5 mM FCA, for 6 minutes, killed 80% of the bacteria cells. The author attests the disinfection is not due to FCA toxicity, instead it is FCA that causes an electrocatalytic oxidation of the intercellular coenzyme A in the bacteria. For actual applications, it would be necessary to somehow immobilize the ferrocene derivative on electrodes, otherwise a flow-through system would require huge amounts of FCA to control biofouling.

The last technique examined (Matsunaga et al., 1998), called a conductive paint electrode, is based on essentially the same principle as the carbon-chloroprene sheet. Carbon black and graphite are the conductive materials. The only difference is that the binder in this application was urethane resin. The advantage of this mixture, over the sheet is easier application to a wider variety of surfaces (even fishing nets). Laboratory tests entailed the use of a conductive paint electrode composed of paint-coated nylon. This electrode was immersed in seawater to allow bacteria to coat the electrode. When a potential of 1.2 V vs. SCE was applied, all of the attached cells were killed. Applying a potential of -0.6 V vs. SCE, resulted in the removal of the attached bacterial cells. Field experiments using coated fishing net yarn, that was immersed for 158 days, showed similar results when an alternating potential was used. Further tests found that the conductive paint electrode was more effective in controlling bacterial growth than was the carbon-chloroprene sheet. The authors concluded that application of an alternating potential, electrically inhibited attachment of biofouling organisms by 94% on the conductive paint electrode, without generating toxic substances.

The conductive paint electrode seems to have the most promise, but many details remain unknown concerning; (1) the application of this method to a large cooling water system, (2) application of this product, to the internal walls of pipes of varying diameters, (3) product durability and effectiveness (as an antifoulant) in a continuous flowing cooling water system for an extended period of time, and finally, (4) the question of cost.

GAMMA IRRADIATION

The engineering problems related to irradiating large volumes of water have never been addressed (Burton & Liden, 1978). Even if this problem was overcome and the method was effective, there would just be too many other complications with such an application. Health and safety issues would be a primary concern, especially for any personnel that had to work in the vicinity of the treatment area. The regulations regarding the use, transport and disposal of any radioactive source are rigid and demanding, therefore, the implementation of such a system would be unpractical and costly.

ULTRAVIOLET RADIATION

The use of ultraviolet-B radiation to control zebra mussel (*Dreissena polymorpha*) infestation has been considered. Results of preliminary work using zebra mussel larvae, indicated an increase in mortality above control levels after 1 hour of exposure (Chalker-Scott et al., 1991). These results were for an experiment conducted in a static, freshwater system, and the results would not be applicable to flow-through systems, where exposure times would be much shorter.

The literature contained one application of ultraviolet radiation as a control of biofouling in heat exchangers (Seki et al., 1985, 1986). Tests utilized four UV units with a maximum dosage of 30 mW-sec/cm^2, and power requirements were 200 W each. Target organisms were bacteria, flow rates used were either 0.91 or 1.83 m/s, and the method produced a 99.5% bacterial kill rate, although the remaining bacteria (0.5%) were able to colonize the surface of the heat exchanger.

In general, the UV radiation method is ineffective in waters containing large amounts organic and inorganic matter, which shields some target organisms from exposure (Burton & Liden,1978; Claudi & Mackie, 1994c). The use of UV radiation prompts health and safety concerns, and to date, the method has been limited to special applications. With regard to the effectiveness of UV radiation in the control of biofouling, concerns need to be addressed: safety issues, effectiveness against a range of biofouling organisms, exposure times required and adaptability to an existing cooling water system.

ULTRASONIC VIBRATION

In a small-scale application of ultrasonic vibration, Kohler & Sahm (1976), investigated the prevention of biofouling of oceanographic sensors. The experiments were carried out in freshwater using a frequency of 40 kHz, and exposure times of 3 to 30

seconds, applied every 1 to 3 hours. After two months, the control sensors were covered with fouling organisms, while the treated sensors remained completely free of fouling. Sensor function seemed unaffected by exposure to the ultrasonic vibrations. Questions surrounding a large-scale application still remain(Burton & Liden, 1978). Only with satisfactory results concerning: effectiveness against all forms of biofouling organisms, effective range, associated noise, effects on equipment and finally cost, could ultrasonic vibration be considered as an alternative means for controlling biofouling.

HYDRAULIC

WATER JET CLEANING

In water jet cleaning, water, under pressure, is used to remove biofouling organisms from surfaces. Generally, the system must be drained prior to cleaning. A wide selection of equipment is available and many large power plants have utilized this method periodically to control mussel infestations. Abrasives can be added to the water to remove residual materials, such as mussel byssal threads. (Claudi & Mackie, 1994c). A drawback with this method is system "down time", and lack of accessibility to inner pipe walls, especially in pipes of small diameter.

WATER VELOCITY

A study conducted in the Netherlands, reported the successful control of mussel infestation via reduced water velocity (Jenner, 1983). Mussel larvae have a relatively slow sinking rate, which is aided by water movement. If intake pipes are long enough (i.e.1200m), and the water velocity low enough (≤ 0.5 m/s), mussel larvae sink before traveling the distance of the intake pipe, and smother in the silt generally contained in these intake pipes. By contrast, increased flow rate prevents larval settlement. A minimum water velocity of 1.5 m/s is required to inhibit settling and attachment (Burton & Liden, 1978; Jenner et al., 1993; Claudi & Mackie, 1994c).

If water velocities can be controlled and the system can tolerate a certain level of biofouling, these are effective/practical, and relatively cheap methods, alleviating the need for additional equipment or chemicals. There are no health or safety risks involved, and the approaches are completely harmless to most organisms and the environment.

MECHANICAL

AMERTAP

The product, Amertap, is a mechanical tube cleaning system designed to provide continuous mechanical cleaning of condenser and heat exchanger tubes. The objective, to keep tubes clean while the unit is in operation, is achieved with specially designed rubber balls that constantly circulate throughout the unit (Drake, 1977). The balls are slightly larger in diameter than the tube, therefore they are compressed as they travel through the tube, and the rubbing action keeps the inside of the tube clean. The balls can be designed for each particular installation to provide the maximum cleaning effect, by altering the level of abrasiveness. Their specific gravity is matched with the cooling medium to obtain a homogenous distribution throughout the system. The balls are circulated in a closed loop, and are caught in a screening device at the discharge end of the condenser tubes. They are then rerouted through a collector unit back to the condenser inlet, where a ball injector controls uniform distribution over the inlet pipe cross-section. The cycle repeats itself, without ever interrupting the operation of the cooling water system.

A similar product, mechanical "pigs" or scrubbers, have been successfully used to remove mussels and other debris from larger diameter pipes (Claudi & Mackie, 1994c). The pigs are available in a wide variety of designs to fit most needs. They are manually forced through the pipe and require system shutdown. Concerns regarding damage to structures due to pressure generated by the mechanical pig on the pipeline have been expressed. To use this product, pipe diameter must remain uniform.

AMERICAN M.A.N.

Like the Amertap system, American M.A.N. is an automatic manual condenser cleaning system which is used during normal operation of the cooling system, and has been found to maintain the efficiency of condensers. In this system, based on the simple principle of flow-driven brushes, cleaning is achieved by passing the abrasive brushes through the condenser tubes by intermittently reversing the flow of the condenser cooling water. Returning normal flow drives the brush back to the original position. Between cleaning cycles the brushes are housed in baskets attached at both ends of each tube in the condenser. The brushes themselves require periodic cleaning because they can become fouled during the cleaning cycles (Burton & Liden, 1978).

The same principle of cleaning, using flow-driven brushes, has been applied to pipe cleaning in cooling water systems. The design is the same as the American M.A.N. system, with flow-driven brushes stored on either end in baskets. The main difference is that this system requires reversing the flow in the whole system (rather than just the

condenser); this can not be done during normal operation. This brush system can only be utilized in straight piping (Santhakumaran, 1989), which excludes its use in cooling systems with extensive bends and diameter changes in the piping.

PIPE ROBOT

The invention of a pipe-crawling robot was motivated by the need to control zebra mussel (*Dreissena polymorpha*) infestation (Landsberger & Martin, 1992). A robot was designed for use in large, land-based power stations that require massive quantities of cooling water. The robot, through self-propulsion, traveled along a fixed cable that was installed through the cooling water system, and cleaning was accomplished through video-controlled, high pressure water jets. Mussels and debris were removed by a suction system similar to dredging equipment, and the robot was remotely operated (via an attached power cable). The robot had wings for buoyancy, wheels for turning corners, and was able to pass through pipes of various diameters. The prototype was built and successfully tested, but trial data and results are not available yet.

MISCELLANEOUS

This section contains all the topics relevant to existing or developing methods for biofouling control that did not fit in any other category, but are topics of interest, regarding the present knowledge in the control of biofouling. Some are not methods in themselves, but play a large role in an existing or developing method.

ANOXIC WATER

Mussels acquire oxygen directly from water and can be deprived of this essential element in several ways: (1) addition of an oxygen scavenging chemical in a closed system, and (2) shutting down the system and allowing the water to go anaerobic for a period of time. The time required depends on the species, concentration, size and/or age of the fouling organisms, and the water temperature. Claudi & Mackie, (1994), reported that, if ambient temperatures are low enough, freshwater zebra mussels (*Dreissena polymorpha*) can survive up to 2 weeks without oxygen in the water.

Mussel (*Mytilus edulis*) larvae have also been tested for their resistance to anoxic waters. Larval resistance is somewhat life stage dependent, but the survival range is 14 to 39 hours (Wang & Widdows, 1991). Adults are more resistant, because of their ability to close and rely on the oxygen (in the water) trapped within their shells. Data for resistance of the adult blue mussel to this treatment were not available.

If the cooling water system in question had a second back-up intake system, this treatment could be used without a long shutdown period. Otherwise, the system shutdown could be prohibitively long, therefore, only cooling water systems with a second intake could realistically consider this as a potential treatment alternative.

LOW ENERGY COATINGS (Table 6)

The term "low energy" relates to the surface free energy of the coating. The molecules on the surface of the coating interact with the water and the dissolved compounds there in. The interaction and forces depend on the chemistry of both the solid and liquid involved. Interactions include van der Waals forces and polar interactions, electrostatic and hydrophobic interactions, and hydrogen bonding. For surfaces, these interactions can be expressed thermodynamically as surface free energy or critical surface tension (Callow & Fletcher, 1994). Simply put, low energy coatings do not provide the energy necessary for secure attachment by fouling organisms, and thereby can deter settlement.

Some of the first compounds considered for low energy coatings (for marine applications) were fluoropolymers. They possess a number of advantageous properties such as: exceptional resistance to the effects of water, sunlight and biological organisms; and they are virtually non-wetting, fast draining, anti-adhesive, low friction surfaces (Griffith, 1982). Leitch & Puzzuoli, (1991), examined the effectiveness of a polytetrafluoroethylene (PTFE) coating against freshwater zebra mussel (*Dreissena polymorpha*) attachment. Coated panels were immersed for a period of 13 months in the field. Results indicated that the PTFE coating showed limited or no resistance to mussel attachment compared with controls. With on-going work on these compounds, new formulations are being considered for testing, such as fluoroepoxies and fluoropolyurethanes.

Another group of low energy coatings being considered for antifouling applications are the silicone polymers, also called silicone elastomers. Like the fluoropolymers, the silicone elastomers provide protection through low adhesion. Callow et al. (1986) examined the resistance of several silicone elastomers to marine biofouling. The three groups of silicone elastomers tested were room temperature vulcanized (RTV) elastomers cured with: (1) tin catalysts, (2) platinum, (3) moisture, and were acetyl tipped. Silicone elastomers that were cured with tin had the best results in both the

Table 6: Miscellaneous- Low Energy Coatings

Type/Description	Coating Composition	Properties	Source
fluoropolymers	polytetrafluoroethylene (PTFE)	Very hydrophobic, low adhesion, application problems.	Griffith (1982)
	fluoroepoxies, fluoropolyurethanes, fluoroacrylics	New, similar properties to PTFE, help with application problems	
silicone elastomers	silicone RTV[1] polymers silicone PC[2] polymers silicone MC[3] polymers	All hydrophobic, low adhesion or surface energy properties, RTV's worked best against algal fouling	Callow et al (1986)
commercial coatings (freshwater, field study, name and manufacturer not provided)	biodegradable wax	Limited/no resistance to mussels	Leitch & Puzzuoli (1991)
	polyurethane	Limited/no resistance to mussels	
	ceramic-filled epoxy/polyamine	Limited/no resistance to mussels	
	PTFE	Limited/no resistance to mussels	
	silicone	Excellent resistance to mussels	
	statically charged zinc	Excellent resistance to mussels	
silicone elastomers (field tests)	poly(dimethylsiloxane) (M)	Hydrophobic, poor fouling results	Edwards et al (1994)
	M + silicone oils	Hydrophobic, poor fouling results	
	poly(dimethyldiphenylsiloxane (MP)	Most hydrophobic, great fouling results	
	MP + silicone oils	Hydrophobic, best fouling results	
Wearlon[R] F1-M	silicone-epoxy	Manufacturer claims good antifouling properties, tough and durable	Internet (see ref.)

RTV[1]: room temperature vulcanizing silicone elastomers cured by addition of tin catalysts

PC[2]: platinum cured silicone elastomers

MC[3]: moisture cured acetyl tipped silicone elastomers

laboratory and field trials, although the differences between the three groups was minimal in most cases. Results indicated further consideration of RTV silicone elastomers for antifouling coatings was merited, but silicone elastomers suffered from poor abrasion resistance and tear strength.

Leitch & Puzzuoli (1991) tested the performance of seven different silicone coatings against freshwater zebra mussel attachment. The authors only referred to the silicone coatings by number, no further information was supplied. Field tests were carried out over a period of up to 13 months. Results indicated excellent resistance to mussel attachment for 5 of the 7 coatings. At least one coating suffered blistering problems which led to mussel attachment.

Edwards et al. (1994) examined the resistance of coatings composed of poly(dimethylsiloxanes) and poly(dimethyldiphenylsiloxanes), with and without the addition of silicone oils, to marine fouling. In total, 27 different formulations were field tested for at least one year, with most coatings exhibiting some level of resistance. One of the tested coatings [poly(dimethyldiphenylsiloxane) with added oil] was exceptionally resistant to all forms of marine fouling.

An Internet search provided one company marketing a silicone-epoxy based coating. The product, Wearlon Super F1-M (www.capital.net/com/ecotech/Emarine.htm), is sold by Eccotech, and claims to be durable, tough, non-toxic, and resistant to both impact damage, and to almost all forms of biofouling, in both fresh and saltwater applications.

Other than being resistant to biofouling, silicone based coatings are non-toxic, with no known environmental impact. Application problems (i.e. controlling coating thickness) remain, and cost restrictions must be overcome before use becomes widespread. These applications will be practical for coating the hulls of ships and other large, immersed structures. However, its use in cooling pipes may be restricted to the internal walls of clean, new pipes that can be visually inspected for coating integrity prior to assembly of a system.

NEW PAINT FORMULATIONS

Since the discovery that the traditional antifouling paints, (namely TBTO paints) are acutely toxic to non-target organisms and result in a variety of environmental problems, replacements have been sought. Another primary problem is achieving a controlled, effective rate of release of the antifouling compound in the paint. If the release rate (solubility of antifoulant) is too fast, the concentration of antifoulant required for effective biofouling control, decreases too quickly, resulting in a paint with a limited period of effectiveness. The opposite (slow release rate) produces a paint that is simply

unable to control biofouling. The goal is a paint possessing a release rate that is effective, but does not deplete the antifoulant in the paint too rapidly.

There are two different approaches to controlled release rate: ablative and non-ablative. In ablative paints, the inhibiting compound is released by breakage of hydrolytic bonds between the paint and the inhibitor. These paints are termed self-polishing because, through movement, these bonds are broken and new paint is exposed, thereby sustaining the process (Anon., 1978a). By contrast, in non-ablative paint, the insoluble binder in the paint holds the soluble inhibitor. As the inhibitor, which is dispersed evenly throughout the paint dissolves, it is replaced by underlying inhibitor (Anon., 1978b). Non-ablative paints have also incorporated the inhibiting compounds in microcapsules (i.e. metal microencapsulation) to aid in the control of release rates (Porter & Miale, 1984; Price et al., 1992; Gatenholm et al., 1995). Ablative paint is intended for active ships, since movement is critical for proper surface renewal. Non-ablative paint is designed for ships and structures that are frequently docked or stationary, since movement is not critical for paint performance.

In a laboratory study, Weisman et al., (1992), tested the effectiveness of the two paint types. Inhibitors used in the paints differed and the results were ambiguous. Neither paint was able to sustain a release rate that effectively controlled biofouling. Ablative paint release rate was too slow, and in the non-ablative paint, the inhibitor was too soluble and resulted in a high release rate initially and a rapid decline to insufficiently low rates, which offered little protection. The ablative type offered more promise, non-ablative paint requires further testing with a less soluble inhibitor, while the ablative paint requires a faster hydrolysis of the bonds holding the inhibitor. Currently, release rates and expected lifetime are the main problems limiting the use and effectiveness of these paints. Once the paints become cost effective with negligible environmental effects, the application problems associated with use in cooling water systems will need to be addressed.

OSMOTIC SHOCK

Osmosis refers to the movement of chemical compounds across a semi-permeable membrane. Equilibrium forces govern the movement of these compounds from an area of high, to an area of low concentration. Osmotic shock is accomplished in the marine environment by the introduction of freshwater. This alters the equilibrium, causing the osmotic movement of chemical compounds, resulting in the loss of essential compounds from the mussel tissue. Aquatic organisms are physiologically adapted to live within a certain salinity range, beyond which they cannot survive (due to osmosis). Open ocean seawater generally has a salinity around 35 ‰, however, tolerance range is species-specific. In the case of marine mussels, which are intertidal and therefore exposed to

widely fluctuating conditions, a wide salinity range can be endured (i.e. acclimatization, valve closure).

In an early study, Brenko & Calabrese, (1969), examined the combined effects of temperature and salinity on mussel (*Mytilus edulis*) larvae. The results indicated that, even at the larval stage, the mussel was very tolerant to a wide range (15-40 ‰) of salinities. Similarly, larvae of the Mediterranean mussel (*Mytilus galloprovincialis*) and the Japanese oyster (*Crassostrea gigas*) have wide tolerance ranges. Salinity had little effect on larval survival and growth (His et al., 1989). In a study of the response of adult mussels (*Mytilus edulis*) to decreasing salinity, Davenport (1979) found a similar tolerance. With a sufficient decline in salinity, mussels responded by closing their valves. At extreme salinities, the mussels isolated themselves indefinitely, otherwise they acclimatized to the new salinity levels. Fox & Corcoran, (1957), exposed two marine mussel species to freshwater in an attempt to induce osmotic shock, to control biofouling at power plants. Exposure time required for 100% mortality of adult (7-10mm) *Mytilus californianus* mussels was 48 hours, and 63 hours for *Mytilus edulis*.

With such high tolerances, control of mussel infestation through osmotic shock can only be accomplished by either of two methods. The first would require access to a massive volume of freshwater to use as a coolant replacement for seawater. For most seawater cooling systems, this is not an option because of limited freshwater supplies. The other choice is closing off the systems and filling them with freshwater, which requires an extended shutdown. To avoid any fouling problems, this procedure would be necessary throughout the entire mussel breeding season. For most large seawater cooling systems this would not be a practical solution. If access to freshwater is possible, then this would be a viable solution, provided the existing infrastructure would be unaffected by the different water compositions.

COMBINATION OF TREATMENTS

Increased restrictions on the use of many established biofouling control chemicals have led to new strategies, particularly since the permissible levels of some chemicals are too low to be effective. As a result, some control chemicals are used in combination to maintain effectiveness at lower concentrations. In the case of hydrogen peroxide + iron, the interest is in the synergistic effect of one chemical on the other.

Evans et al., (1986), looked at the possibility of replacing some of the TBTO in paint with nitrogen-containing cationic surfactants. The two compounds tested were 1-dodecylguanidine acetate and 2-dodecylguanidine acetate. Both were tested alone and in combination with TBTO to determine the effects on diatom growth in the laboratory. Levels of TBTO that were equal to the control in inhibiting diatom growth, were mixed with the two test compounds. The respective compounds provided only partial inhibition

of diatom growth tested individually, but in combination with TBTO, the test mixtures provided much better inhibition.

French & Evans (1986), explored the possibility of partial or total replacement of TBTO in antifouling paints with copper or zinc. Paints were self-polishing copolymers (ablative) composed of TBTO and either copper or zinc, or both. Paints were applied to test panels that were immersed in the field (estuary) for periods of up to 15 months. Results showed an improved level of biofouling control with the paints containing TBTO/copper and TBTO/copper/zinc compared to TBTO alone. The TBTO/zinc paint combination controlled biofouling better than TBTO alone, but was not as effective as the copper combinations.

Mayack et al., (1984), compared the effectiveness of chlorine, chlorine dioxide and chlorine with a dispersant for controlling biofouling. The chlorine/dispersant combination was no more effective at controlling biofouling than the chlorine alone. The assumption was that the dispersant enhanced the effectiveness of chlorine in controlling biofouling. Unfortunately, in this case there was no synergistic effect on chlorine by the dispersant, although the biofilm was easier to remove with the addition of the dispersant.

Knox-Holmes (1993), investigated the combination of copper and chlorine to control biofouling. In a macrofouling experiment, the effectiveness of four different treatments: chlorine, copper, copper/aluminum (Cathelco) and copper/chlorine, were compared. The setup consisted of continuously flowing, untreated seawater, with a flow rate of 2 m/s. The concentration of copper was not provided in the copper (alone) treatment, which proved no more effective than the control. For the chlorine treatment, 200 ppb Cl was administered, while the copper concentration was 35 ppb (much higher than the manufacturer recommended) for the copper/aluminum treatment. The copper/aluminum treatment, like copper alone, offered no better protection than the control. The chlorine treatment was much more effective than the control, but was inferior to the copper/chlorine treatment (where trial concentrations were 5 ppb Cu and 20 ppb Cl). Based on the results from the macrofouling experiment, the microfouling experiment compared the chlorine treatment with the copper/chlorine treatment only (omitted copper and copper/aluminum). The concentrations tested were 200 ppb Cl (sodium hypochlorite) administered for 2 h/day for the chlorine treatment, while 5 ppb Cu was combined with either 50 ppb or 20 ppb Cl administered continuously or intermittently (2 h/day). No significant difference between the four copper/chlorine treatments, (all superior to the chlorine alone) were observed. The copper/chlorine treatment was three times more effective than the chlorine treatment alone in controlling microfouling. The author concluded that low levels of copper (5 ppb) and chlorine (20 ppb) in combination, are more effective for controlling micro- and macrofouling than conventional chlorine (200 ppb) or electrolytic copper (35 ppb) treatments. This technology has been successfully tested in the field on the cooling water system of a ship and currently is being used in commercial situations on ships and offshore platforms.

Strauss (1989), reported that a treatment (20 ppb Cl and 5 ppb Cu), whether applied continuously or intermittently, was at least six times as effective as chlorine against macrofouling, and three times as effective against microfouling. The copper could be added as a chemical, like the chlorine, or it could be generated electrolytically, by way of a copper electrode.

The copper/chlorine treatment has a number of advantages over traditional chlorine treatment. The effectiveness of lower doses of copper/chlorine than either chlorine or copper alone has both environmental (less harm to non-target organisms and decreased formation of chlorinated organic compounds) and financial advantages.

INTERNET PRODUCTS

Most of the common products and even some exotic ones can be obtained through companies listed on the internet. Website addresses for these companies or products can also be found in the reference section.

Sodium hypochlorite can be produced through the use of seawater or brine, via an electrochemical process. Equipment for this electrochemical process, is marketed by Exceltec (www.neosoft.com/~exceltec) and is available in several different sizes.

LATA (www.lata.com), markets equipment (MIOX) that operates through a principle similar to the Exceltec equipment. The system is more effective than on-site hypochlorite generators, and utilizes dilute seawater to produce a mixed-oxidant solution (chlor-oxygen species). The systems are designed for simple installation and operation, and automatically adjust to operating conditions. The system is self-diagnosing and can be equipped for remote monitoring, with the capacity to treat millions of gallons of water a day. The systems, which have been thoroughly field-tested by a number of government agencies and universities (Venczel et al., 1997), are competitively priced with chlorination costs and are cheaper than many of the other alternatives. Low operation and maintenance cost, and the minimal training requirements of personnel for proper operation are attractive features.

BIOFILM

Biofilms are discussed briefly in the earlier discussion of the biofouling process, but recent findings justify a further discussion on this topic. The biofouling process is very complex, with new findings occurring frequently.

The terms biofilm, slime-layer, microfouling all refer to the initial stages of biofouling. The process begins, with biochemical conditioning, the moment the object is immersed in seawater. The adsorption of dissolved chemical compounds to the immersed surface is followed by bacterial colonization. The resulting layer or biofilm is sticky (polysaccaride secreted by bacteria), with the capacity to trap other particles contained in the seawater, and provides the foundation for further colonization by unicellular eukaryonts (i.e. yeasts, protozoa, diatoms). The process is then completed through the colonization by multicellular eukaryotes, which include the invertebrate larvae and algal spores (Field, 1981; Wahl, 1989). There seems to be a distinct, but overlapping, sequence to the biofouling process, with interaction between the different stages.

Kirchman & Mitchell, (1981), studied the effects of biofilm on the settlement and metamorphosis of the invertebrate larvae, *Janua (Dexiospira) brasiliensis*. They found that larval settlement and metamorphosis is a lectin-mediated process: the larvae contain the lectin while the bacterial film produces the carbohydrate (polysaccharides) lectin-receptor. Biofilms composed of a single bacterial species did not always induce larval settlement and metamorphosis. However, it was determined that lectins were involved in larval settlement and metamorphosis, since the addition of glucose (to the seawater) significantly inhibits larval metamorphosis of *Janua*, on surfaces coated with the marine bacterium *Pseudomonas marina*. Lectins were also involved in the settlement and metamorphosis of three species of bryozoans (Kirchman & Mitchell, 1983).

In a later publication, Holmstrom & Kjelleberg, (1994), implied that invertebrate larval settlement was dependent on two factors or stimuli: surface wettability and bacterial-organic film, with the film having greater effect. The mechanisms by which a biofilm can promote macrofouling were categorized as passive entrapment, metabolite and inducer amplification, microbial modulation, and direct attraction and induction.

Satuito et al., (1995), investigated the effects on attachment and metamorphosis of mussel (*Mytilus galloprovincialis*) larvae by microbial film. Pediveliger larvae undergo metamorphosis in response to microbial filmed surfaces and the film does not necessarily need to be alive. Most larvae exposed to a microbial film immediately began to crawl, while control larvae (no biofilm) continued to swim. Larvae that were exposed to autoclaved (dead) microbial films demonstrated behavior similar to that of the control larvae, while formalin-treated biofilm induced larval crawling. A chemical signal(s) may diffuse/leach from the film to induce the crawling/searching behavior of the larvae. In a mussel (*Mytilus galloprovincialis*) study, the microbial film produced a larval inducing factor which remained active after treatment with 5% formalin, but was destroyed by heat (Satuito et al., 1997).

Results strongly suggest a direct link between invertebrate larval settlement/metamorphosis and microbial film (biofilm). In the absence of biofilm, invertebrate larvae may not settle, thereby eliminating the biofouling problem.

One publication (Efird, 1976), studied the relationship between corrosion and fouling of different alloys. Corrosion products influenced the biofilm that was formed on the alloy, indicating that copper/nickel (specifically 90/10 and 70/30) alloys cycled between two different types of biofilm. Copper-base alloys appear to alternately lose and regain their fouling resistance. The initial film formed, which is resistant to fouling, contains cuprous oxide. This oxidizes after a period of time to a greenish cupric hydroxychloride, which is not as toxic to marine organisms, therefore, settlement begins. This second stage (cupric hydroxychloride) is not tightly bound to the surface and is easily removed, preventing secure attachment of the fouling organisms. The sloughing off of the second stage, exposes the more adherent cuprous oxide based film which is more resistant to fouling. This cycling of corrosion products causes a fluctuation between increased and decreased resistance to fouling.

This alternating pattern between high and low resistance to fouling may be influencing the overall biofouling problem. This response of copper-based alloys to biofilm cycling, should be investigated prior to making any decisions regarding treatment.

SUMMARY

The blue mussel (*Mytilus edulis*) is a resilient marine organism, thriving in areas of drastically changing environmental conditions, throughout it's life cycle. Although mussels are considered sessile, the larval/juvenile stages, provide the means for transportation (sometimes great distances). Leading to mussel infestation problems for seawater users', in particular, the piping of cooling water systems. Many different treatments (chemical and nonchemical) have been employed, with various results, to combat these infestations. For a cooling water system, some treatments could be ruled out for practical (i.e. gamma irradiation, paint/coatings), environmental (i.e. Hg salts, tributyltin) and/or financial (i.e. iodine, heat treatment) reasons.

Prior to treatment selection and application the following factors should be considered: (1) biological considerations (i.e. population/age of mussels, discharge site, water temperature and chemistry), (2) physical and chemical factors affecting operation (i.e. size and length of piping, treatment compatibility with pipe material), and (3) economic and logistical considerations (i.e. safety of workers and public, treatment and regulatory costs)(Claudi & Mackie, 1994a).

The industry standard is still chlorine, although regulations are curtailing widespread use in biofouling applications. Chlorine should still be considered because of the wealth of knowledge available, and because of its proven effectiveness and affordability. Preliminary results on the effectiveness of hydrogen peroxide + iron and

potassium ferrate merit some consideration, but not before other factors such as cost, availability, equipment effects, etc., have been investigated. The remainder of the oxidizing agents discussed were less effective and/or more costly than chlorine. Some of the chemicals had health, safety and storage related problems, while for most, the necessary information regarding biological and environmental effects, is lacking.

Most of the nonoxidizing chemicals could not be used in a flow-through, cooling water system, because of their toxicity to non-target organisms and the environment. Use of most of these chemicals (at effective concentrations) would require some form of neutralization prior to discharge, rendering them too costly for use. The only group of compounds that seem to have potential for cooling water systems, are the polyquaternary ammonia compounds, provided they prove effective and affordable. However, questions concerning toxicity to non-target organisms and the environment remain, including information on the long-term effects of most of these chemicals.

Next are the metal salts, of which only copper and mercury seem effective (at reasonable concentrations) in controlling mussel infestation. Mercury could not be considered based on regulations regarding use and discharge. Copper alone can be effective, but the concentrations required to control mussel infestation impose too many negative effects on other organisms and the environment.

The natural products discussed hold enormous potential for combatting biofouling in the future. Unfortunately, none of the products have been developed to a stage where practical applications are possible. If treatment could be delayed for several years, a natural product (in a useable form) may become available. Currently, none of these products warrant serious consideration.

The section on paint additives contains some interesting compounds, but application constraints, make consideration of these compounds unrealistic. If a proven method of application existed for coating internal pipe walls uniformly, these compounds could be seriously examined. Without this application method, paints and low energy coatings could not be considered as an alternative to existing methods.

For this particular biofouling problem, nonchemical methods likely provide the most possibilities. The first treatment, introducing thermal or heated water, into the system, is a simple treatment that offers many advantages: low (or no) cost, highly effective, few negative effects to equipment or the environment. The one requirement is that the cooling water system be designed for such treatments, otherwise equipment alterations can be costly. If the system in question is capable (or can be made capable at a reasonable cost) of thermal treatment, it should definitely be considered.

By comparison, the treatments employing nonthermal energy are, for the most part, impractical, unproven and/or costly. Electrochemical methods using the carbon-chloroprene sheet and the conductive paint electrode hold some promise, but, at present

Table 7: Summary

Chemical Agent/Method	Page No.	Mode of Action	Effectiveness	Deficiencies	Ease of Handling	Hazards	Cost
Chlorine	9	Oxidation	Proven	Environmentally unfriendly, toxic to non-target organisms.	Liquid - good Gas - troublesome	Several / many	Inexpensive
Hydrogen peroxide + iron	20	Oxidation	Unproven	Unknown equipment and non-target organism effects	Good	Few	More expensive than chlorine
Potassium ferrate	22	Oxidation	Unknown	Availability, unknown environ. non-target organism effects	Good	Few	Very expensive
Polyquaternary ammonium compounds	25	Undetermined	Unproven	Unknown environmental and non-target organism effects.	Good	Few	More expensive than chlorine
Metal salts (ie. Hg, Cu)	33, 37	Several possibilities	Proven	Environmental accumulation, toxic to many marine organisms.	Good	Few	Expensive / very expensive
Natural products	44	Undetermined	Unproven (good potential)	Unknown environmental effects, very limited supply.	Unknown	Unknown	Extremely expensive
Paint additives / low energy coatings	47, 61	Various (ie. non-stick surface)	Proven	Application problems and/or environmentally unfriendly.	Paint - good Coatings - fair	Several	Very expensive
Thermal treatment	51	Elevated water temperature	Proven	Slight environmental effect, special equipment requirements	Very good	Few / none	Much cheaper than chlorine
Electrochemical	55	Oxidation / reduction	Partially proven	Application and durability problems.	Unknown	Few	Expensive / very expensive
Water velocity	58	Increased velocity prevents settlement	Proven	Virtually none	Very good	None	Much cheaper than chlorine
Osmotic shock	64	Decreased salinity	Proven	Unknown equipment effects and freshwater requirements.	Very good	None	Much cheaper than chlorine
Copper / chlorine combination	65	Oxidation and/or other(s)	Partially proven	Environmentally unfriendly, toxic to non-target organisms.	Good	Few	Similar to cost of chlorine alone
MIOX	67	Oxidation	Unproven (good potential)	Unknown equipment and environmental effects.	Unknown	Unknown	Unknown

both these treatments suffer from application and durability problems, which eliminates them as potential solutions to our specific biofouling problem.

The simplest and most affordable control treatment would be increasing water velocity. If control of flow rate is possible with the cooling water system in question, this would be the treatment of choice. It does not suffer the drawbacks of other treatments and incurs virtually no negative effects on non-target organisms and the environment.

If increasing the system flow rate is not possible, osmotic shock appears to be an effective method of controlling mussel infestation. The treatment requires specific equipment design and a large supply of freshwater. Osmotic shock has all the advantages of increasing water flow rate, except it would require longer treatment times, with the potential for negatively affecting equipment.

The combination treatments offer one good alternative; the copper/chlorine treatment. The nonchemical treatments would still be preferred since there are fewer concerns generally, about non-target organisms and the environment. If chemical treatment is the only affordable alternative, dosing with copper and chlorine at low concentrations should certainly be investigated. Of all the chemical treatments discussed, other than simple chlorination, it appears to have the most potential as a possible solution to the biofouling problem.

The last suggestion would be the product described on the Internet, MIOX. It is actually a chemical treatment, but seems (based on company information) to be more effective with less undesirable side-effects than chlorine. Before serious consideration is given to this treatment, more information (i.e. installation, effectiveness, marine applications) would be required.

The last point to contemplate before making any decisions would be the role of microbial films (biofilms) in the biofouling process. Treatment for mussel infestation may not be necessary if the biofilm formation is eliminated. The mussel infestation may be simply a product of the presence of a biofilm.

This report was written to provide insight into the complexity of the biofouling process. Key points that need to be considered to properly assess the biofouling situation, prior to selection of the control method have been outlined and the treatments available, with their corresponding advantages and disadvantages have been described and comprise the first steps in finding the proper solution to specific biofouling problems.

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PRODUCTS

Biocide (bromine) M-725: www.magnacheminc.com/products/m725.html

Biocide (glycol) PCT 3015: www.prochemtech.com/chem/proc/shear/3015/3015.html

Exceltec system: www.neosoft.com/~exceltec

List of Antifoulants (cyano, carbamate): www.cdpr.ca.gov/cgi-bin/label/labchemrep.pl

MEXEL 432: www.mexel.fr/mexel432.htm

MIOX system: www.lata.com

Wearlon Super F1-M: www.capital.net/com/ecotech/Emarine.htm

Zinc Pyrithione: www.olin.com/pr13.html

COMPANIES

Chemical Supply: www.calgon.com

www.economypolymers.com

www.elf-atochem.com

General Site for Companies & Products: www.wateronline.com

US Patents: www.uspto.gov/patft/index.html